Universidade de Aveiro
Departamento de Biologia
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Filipa Alexandra Veiga Sobreira

EFEITOS DO RUÍDO GERADO POR NAVIOS NAS VOCALIZAÇÕES SUBAQUÁTICAS DOS GOLFINHOS-ROAZES, *Tursiops truncatus*, NO ESTUÁRIO DO SADO

EFFECTS OF VESSEL NOISE ON UNDERWATER VOCALIZATIONS OF BOTTLENOSE DOLPHINS, *Tursiops truncatus*, IN THE SADO ESTUARY

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha, realizada sob a orientação científica da Doutora Catarina Eira, equiparada a Investigadora Auxiliar do Departamento de Biologia da Universidade de Aveiro, e do Doutor Manuel Eduardo dos Santos, Professor Associado do Departamento de Biociências do ISPA - Instituto Universitário de Ciências Psicológicas, Sociais e da Vida.

o júri

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palavras-chave

Tursiops truncatus, comportamento acústico, ruído de embarcações, sinais vocais, paramêtros acústicos

resumo

O tráfego marítimo é uma fonte de perturbação importante para os cetáceos costeiros, especialmente para populações locais e residentes, como a população de golfinhos-roazes (*Tursiops truncatus*) no estuário do Sado. O ruído provocado por embarcações pode mascarar sinais importantes, como os de comunicação. Para compensar os efeitos de mascaramento, os animais podem modificar o seu comportamento acústico alterando a taxa de emissão, a intensidade do sinal, o tipo de vocalização, a frequência e a duração dos sinais acústicos. Para avaliar os potenciais impactos no comportamento acústico desta população, foram analisados a abundância e as características acústicas dos assobios, dos sinais de ecolocalização e dos sons pulsados em relação ao tráfego de embarcações. As amostras utilizadas foram obtidas através de gravações subaquáticas realizadas na região do estuário do Sado, feitas de março de 2014 a abril de 2017. Os barcos foram considerados como presentes ou ausentes, tendo em conta um raio de 1000 m. Os elementos vocais foram classificados de acordo com as características visuais gráficas e auditivas em: assobios, trens de cliques, buzzes, rangidos, chorincos, trens de taxa variável, bangs, goles, guinchos e grunhidos. A análise das taxas de emissão baseou-se no número de unidades reconhecíveis por minuto para todos os elementos vocais. Na presença de embarcações, não existiram diferenças significativas para as taxas de emissão de todos os tipos de elementos vocais. Para elementos vocais selecionados, foram examinados diferentes parâmetros acústicos, utilizando uma MANOVA não paramétrica, e foram encontradas alterações entre presença e ausência de navios, para as seguintes vocalizações: assobios (X^2 (7) = 56,42; N = 620; p <0,001), rangidos (X^2 (8) = 19,53; N = 94; p = 0,012), grunhidos (X^2 (8) = 80,968; N = 339; p <0,001), goles (X^2 (7) = 58,76; N = 260; p < 0,001) e guinchos (X^2 (10) = 25,894; N = 121; p = 0,004)). Estes resultados mostram modificações no comportamento acústico na presença de embarcações, revelando que os golfinhos-roazes desta população poderão ajustar as suas frequências vocais e produzir sinais mais curtos para manter a comunicação. Este estudo sugere que, embora os golfinhos-roazes residentes do estuário do Sado possam apresentar alguma tolerância ao ruído gerado por barcos no seu habitat, este provavelmente causa mudanças significativas nos seus comportamentos de comunicação.

keywords *Tursiops truncatus*, acoustic behavior, vessel noise, vocal signals, acoustic parameters

abstract Maritime traffic is an important source of disturbance for coastal cetaceans, especially for local and resident populations, like the bottlenose dolphins (*Tursiops truncatus*) population in the Sado estuary. Vessel noise might mask important signals such as communication calls. To compensate masking effects, animals may change their vocal behavior by shifting vocal rate, call intensity, call type, call frequency and duration. To evaluate the potential impacts on the acoustic behavior of this population, abundance and acoustic characteristics of whistles, echolocation signals and burst-pulsed sounds were analyzed in relation to boat traffic. The samples used were obtained in field recordings of dolphin vocalizations made from March 2014 to April 2017. Boat traffic operating within a 1000 m radius was listed as absent or present. Vocal elements were classified according to visual graphical and aural characteristics in: whistles, slow-click trains, short-burst pulses, creaks, squawks, variable rate click trains, bangs, gulps, squeaks and grunts. Analysis of emission rates was based on the number of recognizable units per minute for all vocal elements. In the presence of vessels, differences in call rates were not significant for all types of vocal elements. For selected vocal elements, different acoustic parameters were examined, using a nonparametric MANOVA, and modifications between vessel presence and absence were found for the following vocal elements: whistles (X^2 (7) = 56.42; N = 620; $p < 0.001$), creaks (X^2 (8) = 19.53; N = 94; $p = 0.012$), grunts (X^2 (8) = 80.968; N = 339; $p < 0.001$), gulps (X^2 (7) = 58.76; N = 260; $p < 0.001$) and squeaks (X^2 (10) = 25.894; N = 121; $p = 0.004$)). These results show modifications in acoustic behavior in the presence of vessels, suggesting that bottlenose dolphins in this population might adjust their vocal frequencies and produce shorter signals to maintain communication. This study shows that although resident bottlenose dolphins in Sado estuary seem to display some tolerance to the noise generated from boats in their habitat, it probably causes significant changes in their communication behaviors.

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INTRODUCTION

1. Bottlenose dolphins

The common bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821), is a member of the mammalian order Cetartiodactyla, infraorder Cetacea, parvorder Odontoceti and family Delphinidae (Wells & Scott, 2009).

The widespread use of this dolphin in oceanaria makes it one of the best known of all marine mammals (Shane *et al*., 1986).

Common bottlenose dolphins are primarily a coastal species but may also be found in pelagic habitats throughout tropical to temperate waters, having a worldwide distribution (Reynolds III *et al*., 2000; Würsig & Pearson, 2015). Bottlenose dolphins are cosmopolitan in distribution and demonstrate a great deal of geographical variation in morphology (Wells & Scott, 2009).

Bottlenose dolphins have a robust fusiform body with a tall falcate dorsal fin and long pectoral flippers and colored dorsally light gray to black and laterally with a light belly (Figure 1) (Connor *et al*., 2000; Reynolds III *et al*., 2000; Wells & Scott, 2009). In addition, they have a characteristic sharp demarcation between the melon and the short rostrum (Reynolds III *et al.*, 2000). Adult bottlenose dolphins are large-sized, reaching at least 2 meters to nearly twice that length and can weight 275 kilograms, and in some populations males grow to be larger than females (Reynolds III *et al.*, 2000). Determining the sex of individuals is difficult because there is no pronounced sexual dimorphism, and genitalia and mammary slits are usually inconspicuous (Connor *et al.*, 2000; Reynolds III *et al*., 2000).

Figure 1- Bottlenose dolphin in the Sado estuary (Photo: Patrícia Rachinas-Lopes).

Bottlenose dolphins can live for several decades, reaching 50 years (Reynolds III *et al*., 2000). In general, females reach sexual maturity at 5 to 13 years (Reynolds III *et al*., 2000; Wells & Scott, 2009). After a 12-month gestation period, females give birth to a single calf that remains with its mother for several years (Connor *et al*., 2000; Reynolds III *et al*., 2000). Dolphins as placental mammals have mammary glands and produce milk, which is the primary source of nutrition during the first year of life, when the calf is highly dependent on the mother (Reynolds III *et al*., 2000; Wells & Scott, 2009). Although births have been reported all year, there is a tendency to be seasonal with peaks during spring and summer months (Wells & Scott, 2009).

Bottlenose dolphins feed on a wide variety of prey, taking advantage of any available food source, including a large variety of fish, shellfish and cephalopods (squid, octopus and cuttlefish) (Connor *et al*., 2000; Reynolds III *et al*., 2000; Teixeira, 1982 *in* dos Santos, 1998) and occasionally shrimp and small rays (Connor *et al*., 2000). Across populations dolphins may appear to be generalists but individuals within the population may show some degree of specialization in prey type (Wells & Scott, 2009). Bottlenose dolphins have many strategies for feeding, depending on habitat, group dynamics and prey type, and different populations have developed different feeding methods (Reynolds III *et al*., 2000).

Dolphins are very social animals that form small groups, which coordinate activities (Shane *et al*., 1986; Würsig & Pearson, 2015). Bottlenose dolphins are involved in activities such as foraging, cooperative feeding, resting, travelling, group defense and social behaviors (mating and playing) which are a major component of the dolphins' daily activities (Reynolds III *et al*., 2000; Shane *et al*., 1986).

Activity patterns and habitat structure are apparently the main factors influencing group size (Shane *et al*., 1986). Group sizes are most commonly composed of 2-15 animals and group composition tends to be influenced by sex, age, reproductive condition and familial relationships (Connor *et al*., 2000; Shane *et al*., 1986; Wells & Scott, 2009). Mother and calf establish a very important bond that can last for many years, even after nutritional independency, the calf continues to need its mother for protection, social development and integration into the group (Reynolds III *et al*., 2000). While long term associations exist in the population (between mothers and calves and social bonds that involve other individuals), the composition and structure of the group varies (Würsig & Pearson, 2015). Bottlenose dolphins live in a highly dynamic fission-fusion society, in which individuals associate in small groups with regular structure (composition and size) (Connor *et al*., 2000). The fission-fusion grouping pattern and sex-specific bonds in bottlenose dolphins are rare in mammals but similar to those in chimpanzees and spider monkeys (Connor *et al.*, 2000).

The species is classified as 'Least Concern' by the IUCN (Hammond *et al*., 2012). Although there are many threats on local populations and several resident populations have shown declines over the last two decades (Augusto *et al*., 2012; Bejder *et al*., 2006; Guerra *et al*., 2014), the species is globally widespread and abundant, and a major population decline worldwide is not expected.

1.1 Hearing

Marine mammals are hearing-centric, in the sense that they use sound as their primary sensory modality (Bradley & Stern, 2008). Marine mammals are highly dependent on sound to assess the environment, and it serves the purposes of communication, navigation and exploration, foraging and detection of prey (Bradley & Stern, 2008; Weilgart, 2007). Hearing data is needed to assess the effects that man-made sounds will have on detectability of natural sounds and communication and echolocation sounds (Richardson *et al*., 1995). Odontocetes have evolved a set of structures that functions as a sophisticated underwater bio-sonar system that can provide information (Ary *et al*., 2016).

The hearing system consists of the outer, middle and inner ear (Au, 1993). As in all mammals, hearing capacities are the result of the integrated activity of three fundamental steps: 1) the outer ear captures sound; 2) the middle ear transfers acoustical energy to the inner ear and 3) the inner ear transforms the input into neural impulses (Ketten, 2000). Cetaceans receive sound energy through their lower jaw and send it to their middle ear and inner ear (Au, 1993; Bradley & Stern, 2008). The middle and inner ears are encased in a bony structure called the tympanic bulla, which is connected by connective tissue and fat to the skull (Au, 1993; Au & Hastings,

2008). After sound arrives at the lower jaw, it propagates through specialized “acoustic fat” and is transmitted to the middle and inner ear (Au, 1993; Au & Hastings, 2008; Ary *et al*., 2016). In the inner ear, acoustic energy enters as a compressional wave and causes the basilar membrane to vibrate, deforming the acoustic receptor cells of the organ of Corti and it is transmitted to the brain through the auditory nerve endings (Au, 1993; McCormick *et al*., 1970). Inner ears of dolphins are specialized in detecting and discriminating high frequency sounds, which allows them to have a good frequency discrimination capability (Richardson *et al*., 1995).

Bottlenose dolphins have greatest hearing sensitivity between 40 and 100 kHz (maximum sensitivity 42 dB re 1 µPa), and can respond to frequencies as high as 150 kHz and as low as 40-75 Hz (Au, 1993; Johnson, 1967 in Au, 1993; Richardson *et al*., 1995; Ridgway, 2000).

1.2 Sound production mechanisms

Sound generation in dolphins is important for communication and echolocation (Cranford *et al*., 1996). The sound generation components are located primarily within the enlarged forehead containing mostly the nasal structures (Cranford *et al*., 2015). The sounds are produced in a complex nasal system of air sacs, connective tissue and fat compartments situated in the rostrum termed the “monkey lips”/dorsal bursae (MLDB complex, Figure 2 (Cranford *et al*., 1996). The fat tissue properties and the anatomic geometry contribute to produce a projected beam concentrated forwardly from the melon and provide a mechanism to focus the returning echoes (Ary *et al*., 2016). Vocalizations are produced by the specialized pairs of phonic lips; they are pneumatically driven by air pressure built up in the nasal passages, and propagate through the melon (Au, 1993; Ary *et al*., 2016; Cranford *et al*., 2011). The blowhole opens and closes the dorsal roof of the vestibular sac (Cranford, 2000). The spiracular cavity forms a gap in the floor of the vestibular sac and runs ventrally as a tube and at the extreme dorsolateral margins of the spiracular cavity, the phonic lips complex can be located (Cranford *et al.*, 1996; Cranford, 2000). The laterally broad spiracular cavity can be divided into two functional passageways, each containing one MLDB complex (Cranford, 2000). At the center of each sound generation complex is a pair of small, oblong fatty structures contained within a pair of tough internal lips, the phonic lips (Cranford, 2000). The left and right phonic lips are capable of operating independently or simultaneously being able to produce multiple different sounds at the same time (Cranford *et al*., 1996; Lilly & Miller, 1961; Madsen *et al*., 2013).

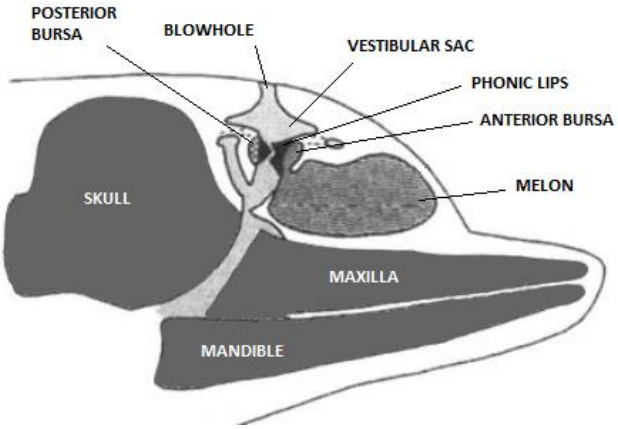


Figure 2- Sound generator: the “monkey lips”/dorsal bursae complex (modified and adapted from Cranford, 2000).

1.3 Communication and echolocation

Cetaceans produce a great variety of vocal and nonvocal sounds (van der Woude, 2009). Nonvocal sounds include sounds produced by percussive activities or produced as a by-product of bodily functions (Cranford, 2000; van der Woude, 2009). Vocal sounds are generated internally and are divided into sound categories, such as tonal vocalizations, "burst-pulses" and echolocation clicks (Luís *et al*., 2016; Richardson *et al.*, 1995; van der Woude, 2009). Tonal vocalizations or whistles are considered to be cohesion calls and communication signals (Janik & Slater, 1998). Click trains are used in sonar-related tasks and detection (Jensen *et al*. 2009; van der Woude, 2009), burst-pulsed sounds are emitted during social interactions, and during foraging/ feeding events (dos Santos *et al*., 1995).

Whistles are tonal frequency modulated calls (with dominant frequencies between 5 - 15kHz) with durations between 0.1 and 4 seconds and can have an ultrasonic range reaching higher harmonics that extend up to 80kHz (Herman and Tavolga, 1980 in Au & Hastings, 2008; Au & Hastings, 2008; dos Santos *et al*., 2005). Short whistles, with maximum duration of 0.1 second are called chirps (Gridley *et al*., 2015). Each individual dolphin develops its unique frequency modulation pattern whistle, termed "signature whistle" that functions as an identifier (Caldwell & Caldwell, 1965; Herzing, 1996; Janik & King, 2013) and may be mimicked by other animals (dos Santos *et al.*, 1990).

Bottlenose dolphins can perceive their environment, by detection, localization, discrimination and recognition of objects by using echolocation signals (Figure 3) (Au, 1993; Tyack, 1997). Echolocation clicks are directional pulsed sounds of high intensity (above 200 dB re 1 µPa @ 1m) and peak frequency (110 - 130 kHz), with very short duration (50 - 200µs) and variable length (Au, 1993; dos Santos, 1995; Richardson *et al*., 1995). During echolocation tasks click trains are usually emitted with inter-click intervals longer than the two-way transit-time, which is the time that an acoustic signal requires to travel from the dolphin to the target and back (Au, 1993).

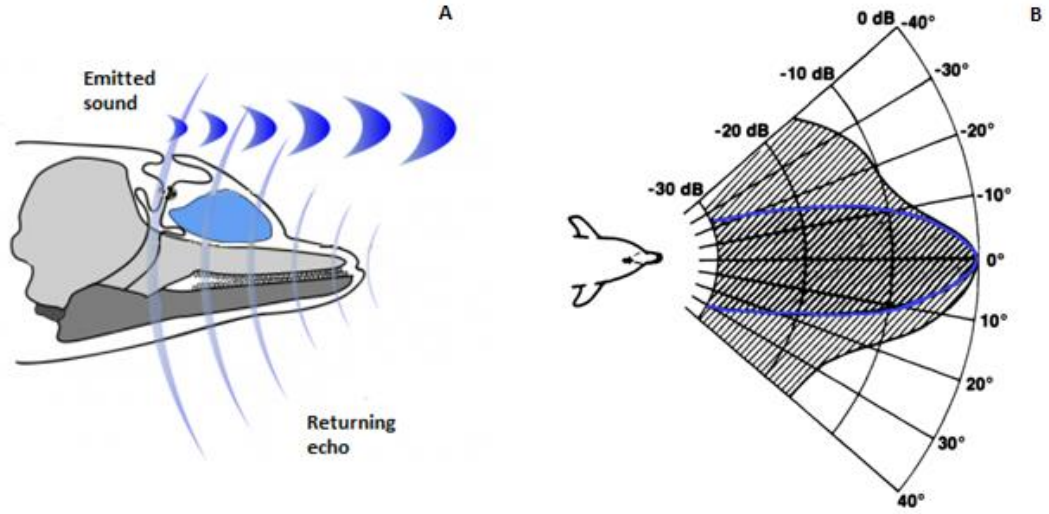


Figure 3- Echolocation in bottlenose dolphin (dark blue is emitted signal from melon and light blue is received echo in the lower jaw) A- modified and adapted from http://us.whales.org/faqs/facts-about-whales-and-dolphins/how-do-dolphins-see-underwater B- modified and adapted from Au, 1993).

Categorization of pulsed sounds is based usually in their aural characteristics and graphical aspects of spectrograms; however, their classification can be problematic because their high-repetition rates can be represented as horizontal bands that resemble harmonics of a tonal sound depending on the analysis window settings (Luís *et al*., 2016; Watkins, 1968).

As the pulse rate increases, click trains change into creaks and short burst-pulse known as buzzes (Luís *et al*., 2014) produced during foraging and feeding events (Herzing, 1996; Miller *et al*., 2004). Although burst-pulses are formed by broadband pulses similar to echolocation signals, they have a high repetition rate (above 300 pulses per second) and short inter-click-intervals (less than 3ms) (Au & Hastings, 2008; Luís *et al*., 2016). Burst-pulses with very high repetition rates (200 - 1200 pulses per second) have been classified as squawks (Herzing, 1996). Bangs have waveforms similar to a typical click waveform, they are isolated high energy pulsed sounds with very short duration (20ms) (dos Santos *et al*., 1990).

The rhythmic sequences classified as bray-series, sound like a donkey's bray and are common in feeding activities and may have a social component (dos Santos *et al*., 1990, 1995; Janik, 2000; King & Janik, 2015). It consists of sequences of squeak-like sounds alternated by grunts and/or gulps (dos Santos *et al*., 1990, 1995). Grunts are broadband burst pulses, with strong emphasis in the lower frequencies; gulps are burst pulse sounds with low-frequency impulsive sounds and squeaks are short pulsed calls with repetition rate faster than time resolution of the spectrogram, that take on an harmonic structure that sounds tonal to the human ear (dos Santos *et al.*, 1995; Luís *et al*., 2014; Watkins, 1967).

1.4 The bottlenose dolphin population of Sado estuary

A resident community of common bottlenose dolphins (*Tursiops truncatus*) lives in the Sado estuary region and it is one of the few resident communities in Europe. Although it is unclear how far back a resident population has inhabited the Sado estuary, their presence was first reported in 1863 and since the 1980's it has been mainly composed of long-term, year-round, resident dolphins (dos Santos & Lacerda, 1987; Gaspar, 2003).

The resident population has been declining during the past three decades. In the past it was estimated at a total of at least 40 and currently there are about 27 dolphins (Augusto *et al*., 2011; Coelho, 2017; dos Santos & Lacerda, 1987). The Sado resident population is one of the smallest coastal resident populations recorded (Gaspar, 2003).

The community size has been declining due to non-existent immigration, low recruitment, low reproductive rates and ageing of the adults (Gaspar, 2003). Additionally, exposure to anthropogenic threats such as pollution from agriculture run-off, industrial and urban non treated sewage, boat traffic, habitat degradation and harassment from dolphin watching and leisure boats, may act to reduce individual fitness or well-being (Cascão, 2001; dos Santos 1998; Gaspar, 2003, 1994). The Sado estuary is exposed to recurrent boat traffic, such as fishing boats, commercial ships, recreational and tourism boats, and regular ferry-boat traffic (Cascão, 2001). The increased and frequent vessel traffic has raised concern of how behavior of this small community of dolphins is being affected (Cascão, 2001; Cruz, 2012; Luís *et al*., 2014). In 2006, a code of conduct was implemented in the Sado estuary, that does not allow the active approach of recreational vessels at a distance of less than 30 m from bottlenose dolphins (Portuguese law 9/2006).

The bottlenose dolphins resident in the Sado estuary have revealed a range of sounds in concordance with the repertoire known for this species: slow-click trains, creaks, squawks, buzzes, bangs, whistles and bray series (dos Santos, 1998; Luís *et al*., 2016).

1.4.1. Previous studies

The Sado estuary is an important habitat for this small resident population of bottlenose dolphins and overlaps with routes of high maritime traffic, which offers an opportunity to study vocal responses of these dolphins to vessel noise. Previous studies in this area have recorded differences in the acoustic behaviour of these animals in the presence of vessels, such as changes in call rate, duration and frequencies of vocal signals.

Luís *et al.* (2014) found differences in mean call of creaks in the presence of some types of boats, as well as changes in whistles characteristics such as shifts in minimum, maximum and start frequencies in the presence of vessels. In the study of Rocha (2012), decreases in whistle emission were also found in the presence of vessels for all activities but had a significant importance in foraging and feeding. Differences in the start and end frequencies were also observed in the presence of boats.

Studies that measured sound pressure levels in the Sado estimated values for all types of vessels and found that higher levels corresponded to bigger ships and were approximately 140 dB re 1 µPa (dos Santos, 1998); as for smaller boats it was lower than 110 dB re 1µPa (Luís *et al*., 2012). Also, the dominant noise bands of vessels vary between 400 Hz e 10 kHz and potential impact of the noise originated noise by the different kinds of vessels is variable (Cruz, 2012). Furthermore, mean call rates of whistles, squawks and creaks were lower in the presence of boats (Luís *et al.*, 2012). Differences in start, end and minimum frequencies and bandwidth of whistles in the presence of vessels were also observed (Luís *et al.*, 2012).

Other studies observed behavioral changes like evasive movements and changes in diving patterns (Luís, 2007) and recorded a decrease in the number of blows per surfacing sequence, and an increase of the dive duration in the presence of boats (Cascão, 2001). That study also found that dolphins in the Sado estuary changed their activity, orientation and group composition or spatial structure and increased the number of tailslaps in the presence of vessels.

2. Acoustic Concepts

Sound may contain information that is useful (a signal) or not useful (noise) (Bradley & Stern, 2008). Two of the quantities most frequently used to describe sound are acoustic pressure and intensity, which are closely related, since it is easier to measure and detect changes in pressure and then convert these to intensities (Bass & Clark, 2003; Simmonds *et al*., 2003). Bioacousticians typically do not measure sound intensity but rather sound-pressure level (SPL), measured in decibel (dB) (Bass & Clark, 2003). Acousticians use ratios of parameters, requiring the use of a standard reference for the denominator, and a logarithmic base 10 scale was adopted and denoted the decibel (dB) scale (Bradley & Stern, 2008; Richardson *et al*., 1995). Due to the difference between the in-air and in-water acoustics, the standard in-water reference pressure is 1 µPa (Bass & Clark, 2003). Acoustic waves are characterized by their frequency (f) which is the number of cycles per second and defined in Hertz (Hz); period (T) which is the duration of a single cycle and measured in seconds; and wavelength (ʎ) which is the distance covered by one full cycle of the sound (Kinsler *et* al., 2000).

2.1 Ambient Noise

Ambient noise is the sound that masks other sounds of interest, and is the result of both natural and anthropogenic sources (Hildebrand, 2009; Richardson et al., 1995). Biological noise arises in the ocean from a variety of animals such as fish, shrimp and marine mammals (Bradley & Stern, 2008; Richardson et al., 1995). Other environmental sources are natural abiotic components such as precipitation, seismic disturbances, underwater volcanoes, wind and waves (Bradley & Stern, 2008). Anthropogenic sources are sounds produced by humans intentionally or as an unintended by-product of their activities (Bradley & Stern, 2008; Shannon *et al*., 2015). For purposes of understanding the sources, ambient noise can be divided into frequency bands: low (10 to 500Hz), medium (500Hz to 25kHz) and high (>25kHz) (Hildebrand, 2009).

2.2 Natural Noise Sources

Natural sources dominate time-averaged ocean noise spectra below 5Hz up to 200 kHz (NRC, 2003). The dominant source of naturally occurring noise is associated with ocean surface waves generated by wind currents (NRC, 2003). Marine organisms such as marine mammals, snapping shrimp and fish use sound to communicate and/or navigate and are an important component of natural noise (Hildebrand, 2009). Marine mammal vocalizations cover a wide range of frequencies, from 10 Hz to 200 kHz, playing a significant role in marine ambient noise (NRC, 2003). Odontocetes, dolphins and toothed whales, produce a broadband of acoustic signals, with different peak spectra, that can be characterized by species (NRC, 2003; Richardson *et al*., 1995). For example, *Tursiops truncatus*, achieve peak energy at higher frequencies in a high-noise environment (Au, 1993). Further, there are many physical natural mechanisms occurring in the water that produces noise and, as such, the ocean is never without sound (Bradley & Stern, 2008).

2.3 Anthropogenic Noise Sources

Noise generated by human activities has increased drastically over the last decades due to population growth, urbanization, globalization and expansion, contributing to ambient noise (Bradley & Stern, 2008; Shannon *et al*., 2015).

There are many contributors to noise in the sea, such as maritime traffic, commercial tourism, sonars, fishing industry, offshore petroleum exploration and exploitation, marine construction works and explosions (Bradley & Stern, 2008; dos Santos *et al*., 2010; Richardson *et al*., 1995). Chemical explosives are used for several purposes underwater, including seismic surveying and construction (Hildebrand, 2009). Due to the high energy level of the shock waves in a very short time, explosions must be considered especially dangerous to marine mammals (dos Santos *et al*., 2010; Richardson *et al*., 1995). Industrial activities such as oil drilling and marine dredging and various construction activities can create underwater sounds (Hildebrand, 2009; Richardson *et al*., 1995). Dredges are used to deepen channels and harbors, being commonly used in coastal waters and can be strong sources of continuous noise in nearshore regions (Richardson *et al*., 1995). Marine geophysical surveys are conducted to study geological processes and locate geological structures, using high-energy short-duration pulses to create seismic waves (Richardson *et al*., 1995). Sonar systems have both military and civilian applications, with a wide range of frequencies. Sonar frequencies range from low to high frequency sonars used in mine hunting, mapping and profiling (Richardson *et al*., 1995). Low-power acoustic deterrent devices (ADD) are used in some fisheries to try to keep marine mammals away from fishing gear or aquaculture facilities (Hildebrand, 2009). The navies have a variety of sonar-based devices, including search sonars, acoustic homing devices for torpedoes, obstacle avoidance sensors, and communication systems (Bradley & Stern, 2008). Mass strandings of beaked whales have occurred in a temporal and spatial association with ongoing

military exercises employing multiple high-energy, mid-frequency (1-10 kHz) sonars (NRC, 2003; Parsons *et al*., 2008). Military sonars can change the whale's behavior, disrupt activities and so can cause directly or indirectly acute tissue damage, and consequently, death and stranding events (Goldbogen *et al*., 2013; Jepson *et al*., 2003).

2.3.1 Vessel Noise

Maritime traffic is an issue of major ecological concern (Jensen *et al.,* 2009). Vessels are major contributors to the overall background noise in the sea, especially at low frequencies between 5 and 500Hz (NRC, 2003; Richardson *et al*., 1995). Over the last years there has been a significant increase in the number and size of individual vessels in the world (Hildebrand, 2009). Vessel noise is an important source of disturbance for coastal cetaceans, especially in the case of local and resident populations, like the resident group of bottlenose dolphins in the Sado estuary (Jensen *et al*., 2009; Luís *et al*., 2014).

The contribution of shipping noise is twofold, the dynamics of a moving ship at great distances is labeled as prevailing noise (constantly present), but as the ship gets closer, its individual contribution arises and becomes local traffic (Bradley & Stern, 2008). The primary sources of sounds produced by vessels are propeller cavitation, propeller "singing", and engines and other machinery (Richardson *et al*., 1995). Cavitation at the propeller blade tips is a significant noise mechanism across all frequencies, and includes both broadband noise due to bubble collapse, and there are also tonal noise bands related with the blade passage frequency (Hildebrand, 2009). The number and type of vessel has impacts on the animal's behavior (Constantine *et al*., 2004). Vessels ranging from the smallest boats to the largest supertankers all produce noise (Richardson *et al*., 1995). Individual vessels produce unique acoustic signatures that can change with ship speed, load and activities taking place on board (Hildebrand, 2009; NRC, 2003). Large vessels create stronger and lower-frequency sounds due to their greater power and slower turning engines (Richardson *et al*., 1995). Commercial shipping is a significant component of ocean ambient noise at low frequencies (Hildebrand, 2009). Peak spectral levels for individual commercial ships are in the frequency band of 10 to 50 Hz and range from 195 dB re µPa2/Hz for fast-moving supertankers to 140 dB re µPa2/Hz for small fishing vessels (NRC 2003). Although the fishing industry makes use of smaller vessels, the number of vessels is larger and it still presents as a major contributor (Bradley & Stern, 2008). Further, fishing vessels also add another source of noise: echo-sounding that requires high frequencies in short tonal pulses and is used for locating fish (Bradley & Stern, 2008; Hildebrand, 2009). In coastal areas, whale and dolphin watching vessels constitute a particular type of vessel traffic that deserves special attention because these vessels approach and chase cetacean groups (Jensen *et al.,* 2009).

2.3.1.1 Effects of vessel noise on marine mammals

In the way marine mammals respond to underwater sounds, reactions can vary greatly within a species and differences between sex and age classes are expected (NRC, 2003; Richardson *et al*., 1995). Evidence suggests that the characteristics of the acoustic signal (frequency, duration, intensity), the hearing range, behavioral state, and habitat are important for predicting how noise affects a particular organism (Shannon *et al*., 2015). Unfortunately, existing data are insufficient to predict accurately acoustic impacts on marine mammals (NRC, 2003).

Short-term effects

Vessel noise might impact cetaceans by masking sounds like communication calls and other important natural sounds (Jensen *et al*., 2009; Richardson *et al*., 1995). Masking is the reduction of an animal´s ability to detect relevant sounds in the presence of other sounds and occurs when frequencies of the signal and masking sound overlap (NRC, 2003). However, there are few data on the consequences of masking for various periods of time (Richardson *et al*., 1995).

Dolphins often tolerate or even approach vessels, but can show avoidance or escape from the region, and reactions seem to be related to the dolphin's activity (NRC, 2003; Richardson *et al*., 1995). Avoidance reactions are the most obvious manifestations of disturbances, since animals swim rapidly away from a noise source (Richardson *et al*., 1995). Most data on marine mammal disturbance concerns short-term behavioral reactions, as such, it is difficult to assess the consequences of a disruption in natural activities (Richardson *et al*., 1995). Bottlenose dolphins commonly approach vessels, swimming in their bow and stern waves showing tolerance to their presence (Figure 4), but boats often cause altered behavior (Richardson *et al*., 1995). Interactions with dolphin-watching vessels have impact on animal's activity (Acevedo, 1991; Arcangeli & Crosti, 2009; Hashim & Jaaman, 2011; Meissner *et al*., 2015). Behavioral response reactions include changes in mean durations of surfacing and dives, number of blows per surfacing and intervals between successive blows (Cascão, 2001; Nowacek *et al*., 2001), variations in group structure (Arcangeli & Crosti, 2009; Guerra *et al*., 2014), and in travelling behavior (Nowacek *et al*., 2001; Acevedo, 1991).

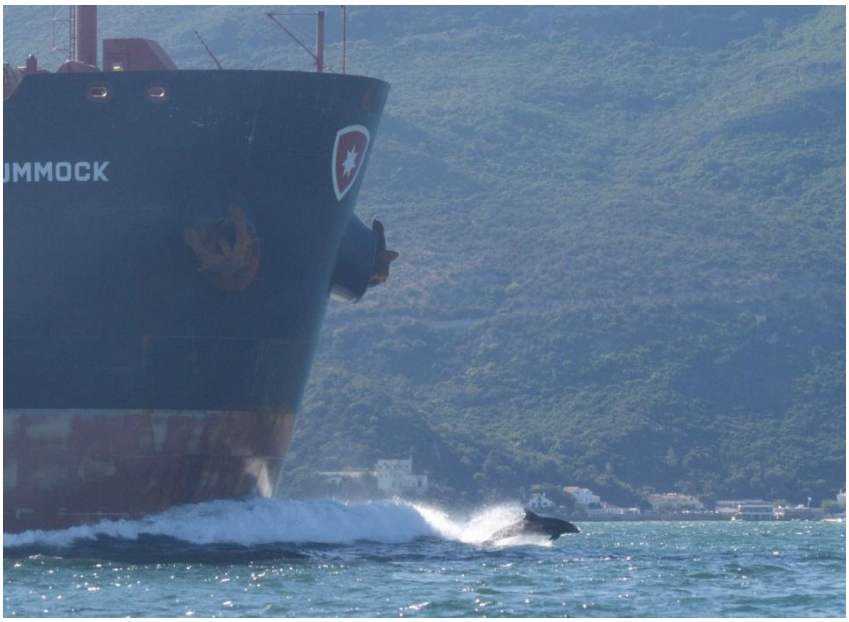


Figure 4- Interaction between bottlenose dolphin and vessel in the Sado estuary (Photo: Cecília Vilhena Ferreira).

An animal may adapt its vocal behavior to compensate for increased levels of masking noise by shifts in vocal rate, call intensity, call type, call frequency and duration (Bittencourt *et al*., 2016; Buckstaff, 2004; Guerra *et al*., 2014; Luís *et al*., 2014). A common reaction of cetaceans exposed to noise is to cease or reduce calling, leading to social disruption (Richardson *et al*., 1995). A potentially severe consequence of disturbance-induced social disruption is the separation of dependent calves from their mothers (Richardson *et al*., 1995). Evidence show that changes in bottlenose dolphins whistle acoustic structure depends on their behavioral state and foraging activities (May-Collado & Quiñones-Lebrón, 2014).

Long-term effects

Stress can be defined as a perturbation to homeostasis. When the perturbation has a short duration, homeostasis is restored through adaptive stress response, but when it is persistent, the stress response can be pathological (NRC, 2003). Although stress-induced pathologies have been hard to identify in marine mammals, based on studies with terrestrial mammals, it is likely that marine mammals would experience the same responses (NRC, 2003).

Exposure to high intensity sound for a sufficient duration may result in auditory effects such as a noise-induced threshold shift (NITS)—an increase in the auditory threshold after exposure to noise. The noise-induced threshold shift may be permanent, called a permanent threshold shift (PTS), or temporary, called a temporary threshold shift (TTS) (Finneran *et al*., 2005). TTS and PTS depend on the level of the sound, its duration, and the mammal (Bradley & Stern, 2008). Long term effects of ocean sounds on auditory abilities may include the transformation of temporary threshold shifts to permanent threshold shifts and increase in occurrence of pathological stress (NRC, 2003). It is believed that permanent hearing impairment caused by prolonged exposure to continuous man-made noise is not likely to occur in marine mammals for sources with levels up to 200dB re 1µPa (Richardson *et al.*, 1995). PTS and TTS may have less obvious effects than shock trauma but they are equally serious, since they can affect communication, breeding behavior, or navigation (dos Santos *et al*., 2010).

Repeated incidents of interrupted feeding or rapid swimming due to disturbance probably have negative effects on the well-being of individuals if disturbance occurs often and for long periods (Richardson *et al*., 1995). Recent studies have argued that short-term behavioral changes can have long-term implications for populations by reducing energy uptake and/or increasing physical demands (Meissner *et al*., 2015). An increase in vessel activity has been linked to long-term declines in dolphin abundance (Bedjer *et al*., 2006). Further, there is increasing evidence that individual behavioral changes can potentially lead to population-level effects (Meissner *et al*., 2015). It is important to notice that population-level effects may significantly alter important characteristics of marine ecosystems because cetaceans are top predators and play a critical role in food-web structure and ecosystem function.

Large amplitudes, as well as, other acoustic effects, can cause bodily harm, including tissue damage, bleeding, organ injury, and death (either directly by the sound or indirectly by the response from the mammal) (Bradley & Stern*,* 2008; van der Meij *et al*., 2015).

3. Thesis objectives

The common bottlenose dolphins of resident population in the region of the Sado estuary are exposed to vessel noise on a daily basis. The principal objectives in this study are:

- Based on field sampling of underwater sound near dolphins groups, to provide a simple characterization of common noise sources on this environment.

- To evaluate the potential impacts on the acoustic behavior of bottlenose dolphins, regarding the abundance and acoustic characteristics of vocal communication and echolocation emissions. Specifically examine overall call rates, whistle characteristics, and whether changes in the various burst-pulsed emissions occur in relation to boat traffic.

MATERIAL AND METHODS

1. Study site

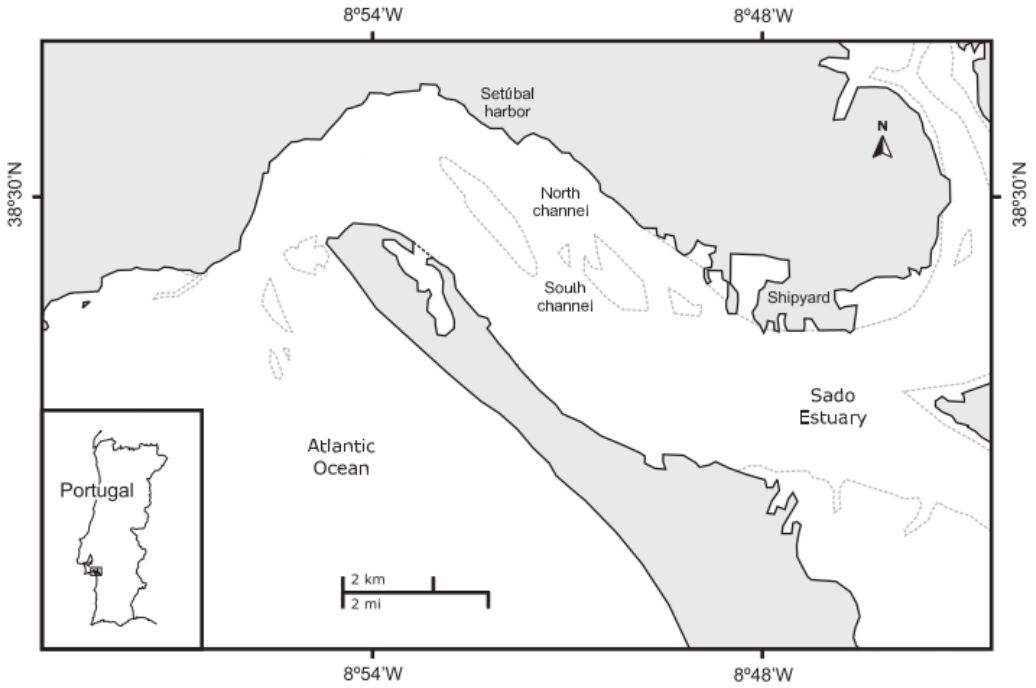


Figure 5- Map of the study area in Sado estuary, Portugal (modified and adapted from Luís *et al*., 2014).

The study was conducted in the Sado estuary and adjacent coastal waters, located on the Western coast of continental Portugal (approximate location 38º 29' N, 8º 55' W, Figure 5). The estuary is divided into two channels separated by sand and mud banks (dos Santos & Lacerda, 1987). The North channel, with a maximum depth of 15 m, is heavily influenced by the city of Setúbal, its harbor and industrial zones (dos Santos *et al*., 2005). The South channel is bordered by the sandy Tróia Peninsula, mainly used for tourism and recreational activities and shows a more intense water flow (dos Santos *et al*., 2005, 2007). Previous studies show that dolphins spend more time feeding in the South channel compared with other areas of the estuary (dos Santos *et al*., 2001). Maximum depths are 40 m at the mouth of the estuary, 25 m in the South channel, and 15 m in the North channel (dos Santos & Lacerda, 1987). To the West of the estuary mouth, bordered by Arrábida, is a coastal area characterized by a rocky shoreline and an important marine park, the Arrábida Natural Park (Sequeira *et al*., 2009). The Sado estuary is the second largest estuary in Portugal and presents a unique variety of habitats and biological richness which led to the creation of The Sado Estuary Nature Reserve in 1980, covering the upper estuary (Neves *et al*., 2004).

2. Field Recordings

All data were collected from an inboard motor research vessel during daylight hours (1000 – 1800), from March 2014 to April 2017, with sea state ranging from 0 to 2 Beaufort. The initial recordings (2014-2016) were obtained in field trips carried out by lab members, using the same procedures as described below.

When vessels were operating within a 1000 m the data collection started. The distance from the vessels was measured from our research boat to each individual vessel, using a NewCon Optik LRM 2000PRO rangefinder.

Whenever a group of dolphins was detected, the research vessel was positioned approximately 500 m ahead of the group's displacement, with the engines and boat power off, and after 15 min (habituation period), the hydrophone was placed at a depth of 3 m to 5 m to initiate the recordings. Dolphins' group size was determined by direct counting of the animals by two observers, averaging the counts. As groups were also photographed for individual identification, group size was also subsequently corrected.

All acoustic measurements were carried out using a factory-calibrated recording system (Figure 6): a Cetacean Research Technology hydrophone, model C55 with effective sensitivity of −165 dB re 1 V/µPa, frequency response of ±3/20 dB in the 0.006-203 Hz band and in the 0.009–100 kHz band, polarized by a 9 V battery, and connected by a 15 m cable to a Fostex FR-2 digital recorder. A high-pass filter of 100 Hz was activated to avoid self-noise generated by the recording platform and low-frequency vibrations. One-minute duration recordings were made, with 5 min intervals to promote independence among samples, with a sampling rate of 192 kHz and 24-bit resolution, recording level at 7.5 and trim level at −26 dB. All digital recordings were stored on Compact Flash memory cards as time-stamped wave files for subsequent signal analysis. The geographic location of each recording was given by a Garmin Foretrex 301 portable GPS.

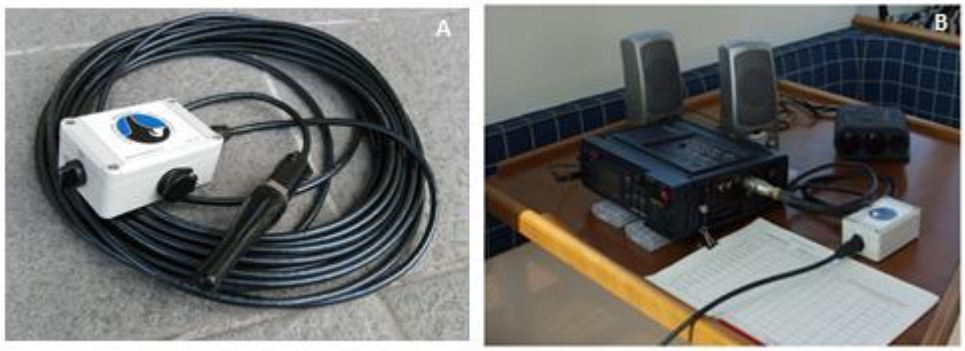


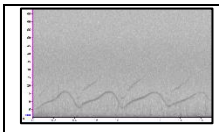
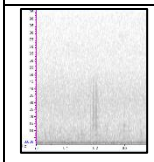
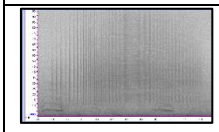
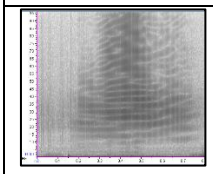
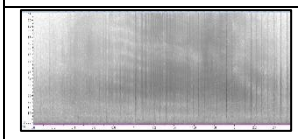
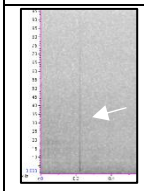
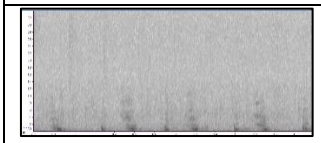
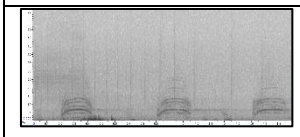
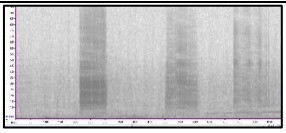
Figure 6- Equipment and electroacoustic instrumentation: A- Hydrophone and B-Fostex digital recorder.

Behavioral context was also recorded according to dos Santos *et al*. (2005) categories: (1) travel—rapid, linear and consistent directional displacement of the group, moving between areas and with no aerial behaviors; (2) foraging—associated with search for food, characterized by zigzag displacement of a subdivided group, with irregular longer dives and occasional aerial activity or fish chase; (3) feeding—individual animals, dyads, or tryads surfacing more than 10 m apart, with very short dives and abundant movements at the surface, including captures, prey leaping, or prey toss; and (4) socializing—dyads or tryads showing excited surface and aerial behaviors, with gentle body contact such as rubbing, and occasional synchronous movements; (5) Resting- tight cohesive group formation with little or no movement at the surface (dos Santos, 1998).

3. Acoustic Analysis

Recordings were first inspected by two trained independent observers, aurally and visually, using the software Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY) with Hann windows of 512 points, frequency resolution of 375 Hz and 50 % overlap, to identify, categorize, and count all the vocal elements present in each sample. Vocal elements were classified to one of the following pre-established categories, according to graphical and aural characteristics as explained in Table 1:

Table 1- Categories of vocal elements or bursts used in emission classification (see more details in Appendix I)

	**whistle:** Tonal, non-pulsed, narrow-band, modulated signals with durations between 0.1 and 4 seconds.
	**slow-click trains:** Discernible click trains with long duration (> 1 s) and repetition rate < 40 pps
	**Short-burst pulses (S-BP):** Short burst-pulse (< 0.2 s), with elevated repetition rate (200-400 pps), aurally similar to a buzzing bee but shorter
	**creaks:** Long burst-pulse (>0.2 s), with repetition rate elevated (40-200 pps), aurally similar to a creaking door
	**squawks:** Long burst-pulse (>0.2 s), with elevated repetition rate (200-600 pps), similar of a crying baby
	**Variable rate click trains (TTV):** Variable rate click trains that may contain discernible clicks, creaks and squawks, with high repetition rate (>50 pps)
	**bangs:** Isolated short pulse signals with high energy
	**gulps:** Short pulsed low-frequency impulsive sounds, similar to a sob
	**squeaks:** Short pulsed calls with harmonic structure that sound tonal, similar to a scream
	**grunts:** Trains of intense burst pulses, with strong emphasis in the lower frequencies, similar to a pig grunt

pps- pulses per second

The analysis of emission rates was based on the number of recognizable units per minute for all vocal elements described in Table 1.

All the identified sounds were rated based on signal-to-noise ratio (SNR) as follows: (i) poor –signal faint and hardly visible on the spectrogram, (ii) fair – signal visible and with a clear start and end on the spectrogram, (iii) good – signal well marked and with a clear start and end on the spectrogram (Luís *et al*., 2016). Non-overlapping signals rated as fair or good where selected for further analysis.

For the selected pulsed signals, the following acoustic parameters were measured: minimum frequency, maximum frequency, frequency range, peak frequency, duration, number of pulses (counted manually using a playback rate of 0.01, with visual inspection of the spectrogram), inter-click interval (ICI; interval between each pulse) and repetition rate (number of pulses per second). Repetition rate (pps) and inter-click interval (ms) were calculated based on the number of pulses and the duration of each sample. Non-overlapping trains of slow clicks were selected and peak frequency, duration, number of pulses, ICI and repetition rate were measured.

In order to allow the best possible description of each sound type, a different number of points in a Hann window were used to examine gulps (3000 points), grunts (256 points) and squeaks (700 points). For these vocal elements, minimum frequency, maximum frequency, frequency range, peak frequency and duration to all sounds were measured. Additionally, other parameters were analyzed for each element: start frequency and end frequency for gulps; number of pulses (counted manually using a playback rate of 0.003), inter-click interval (ICI) and repetition rate for grunts; and for squeaks beginning frequency, end frequency, ICI and repetition rate.

For selected whistles, features as peak frequency, minimum frequency, maximum frequency, duration, start frequency, end frequency and inflexion points were also analyzed.

4. Statistical Analysis

As emission rates could be a direct function of group size, this variable was analyzed. Correlations were calculated between group size and emission rates (Pearson product-moment correlation). A nonparametric Wilcoxon-Mann-Whitney test was performed to compare the emission rate with presence/absence of vessels.

To compare each acoustic parameter of every vocal element with presence/absence of vessels; whistles, squawks, creaks, slow-click trains, squeaks, grunts, gulps; a nonparametric MANOVA as described in Marôco (2011) was used. Whenever a MANOVA showed significance, Wilcoxon-Mann-Whitney tests were used to assess which acoustic parameter was significantly different. All statistical analyses were performed using IBM SPSS Statistics 21 (IBM Inc.)

RESULTS

A total of 161 samples (no boats = 99, boats present = 62) recorded within the study area were selected for analysis.

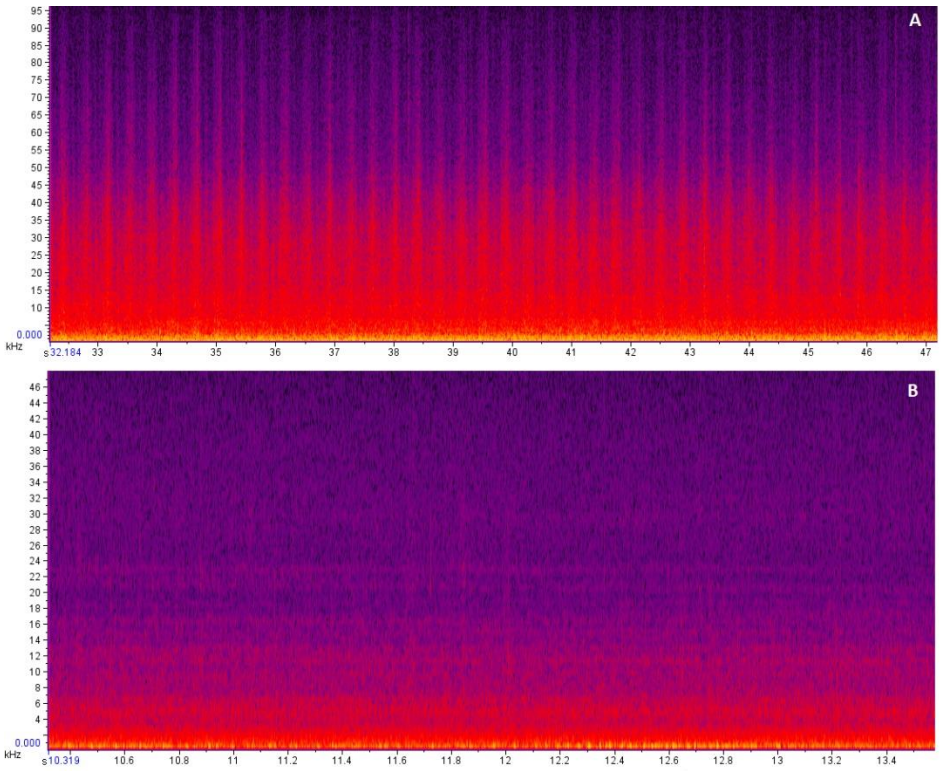


Figure 7- Sonograms of vessels in Sado estuary: A-Large vessel at a distance of 300 m and B- Small vessel at a distance of 1000 m.

In general, all vessels produce noise, from the smallest to the largest (Figure 7). Though, large ships tend to be noisier than small ships. As mention before, the primary sources of sounds from vessels are propeller cavitation, propeller "singing" and engines or other machinery (Richardson *et al*., 1995). Propeller cavitation is usually the dominant noise source, and broadband components caused by propeller cavitation may extend to 100 kHz (Richardson *et al*., 1995). The broadband components are related to propeller cavitation, and the narrow band components are associated with the rotation of the propellers (dos Santos, 1998).

The Setúbal Harbor is the third most important in Portugal, and is located in the Sado estuary. Vessel noise in the estuary is constant, as there is an annual traffic of around 1600 ships in Setúbal Harbor (APSS, 2016). Furthermore, in the summer months, there is an increase in the number of recreational craft circulating in the estuary and dolphin-watching activities (Sequeira *et al.*, 2009; Cascão, 2001).

No correlations were found between dolphin group size and the mean emission rate of each sample ($r = 0.003$; $n = 149$; $p = 0.971$). Therefore, it may be assumed that more dolphins do not necessarily produce more emissions, and so, group size was excluded from the analysis of variance of vocal elements and acoustic parameters.

Kolmogorov-Smirnov tests were used to test for normality for each variable, and assumptions of normality were not met. As such, a Wilcoxon-Mann-Whitney test was performed to compare the emission rate with presence and absence of vessels.

Emission rates

The analysis of means for each type of vocal element showed that differences in emission rates in the presence and absence of vessels were not significant: bangs (U = 2867; W = 4820; p = 0.152); short burst-pulses (U = 3060; W = 5013; p = 0.934); slow-click trains (U = 2921; W = 4874; p = 0.608); creaks (U = 3433; W = 5386; p = 0.112); squawks (U = 3077; W = 5030; p = 0.974); TTV (U = 2984; W = 4937; p = 0.711); whistles (U = 2602; W = 4555; p = 0.082); grunts (U = 2941; W = 4894; p = 0.576); gulps (U = 2863; W = 4816; p = 0.257); squeaks (U =2691; W = 4644; p = 0.053) (Figure 8, for more details see Table 2, in Appendix II).

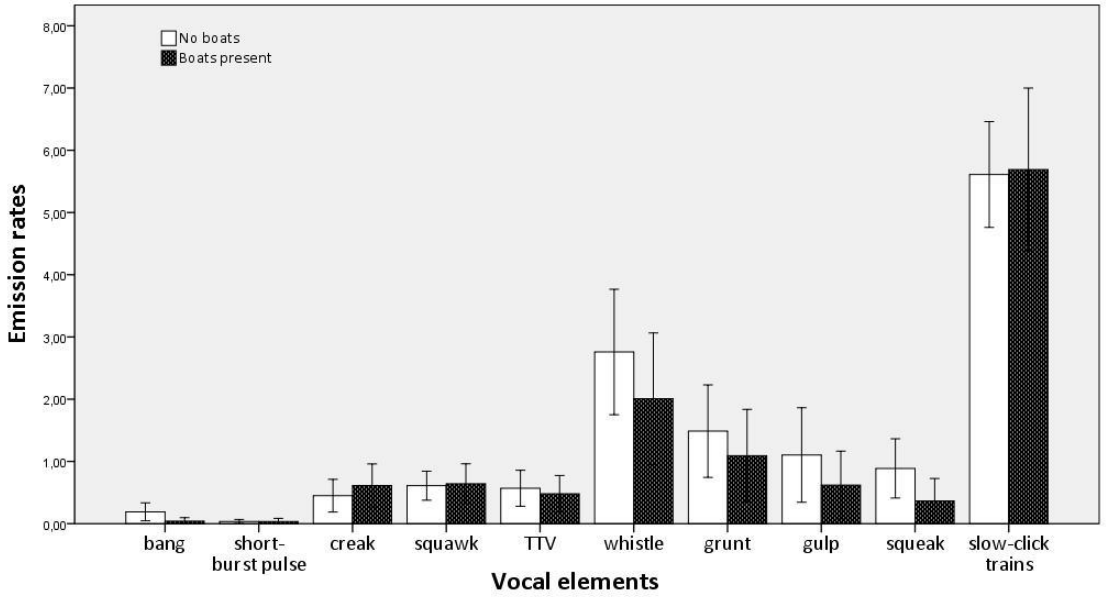


Figure 8- Emission rates of all vocal elements in the presence and absence of vessels.

Normality tests (Kolmogorov-Smirnov) performed for all acoustic parameters of each type of vocal elements, failed to meet the assumption of normality, thus, nonparametric multivariate analyses of variance (NPMANOVA) were used.

Whistles

A total of 621 whistles (no boats = 405; boats present = 216) were selected for acoustic parameter analysis. Nonparametric multivariate analyses of variance showed statistically significant differences in the acoustic parameters between presence and absence of vessels (X^2 (7) = 56.42; N = 620; p < 0.001). Significant differences were found for the minimum frequency (U = 53579; W = 77015; p < 0.001); start frequency (U = 56551; W = 79987; p < 0.001) and duration of whistles (U = 36994; W = 60430; p = 0.002). The minimum and start frequency of whistles in the dolphin population of the Sado estuary were significantly higher in the presence of vessels than in their absence (Figure ,). The duration of whistles was longer in absence of vessels than in their presence (Figure). Differences in peak frequency, maximum frequency, end frequency and inflexion points were not significant (for more details, see Table 3, in Appendix II).

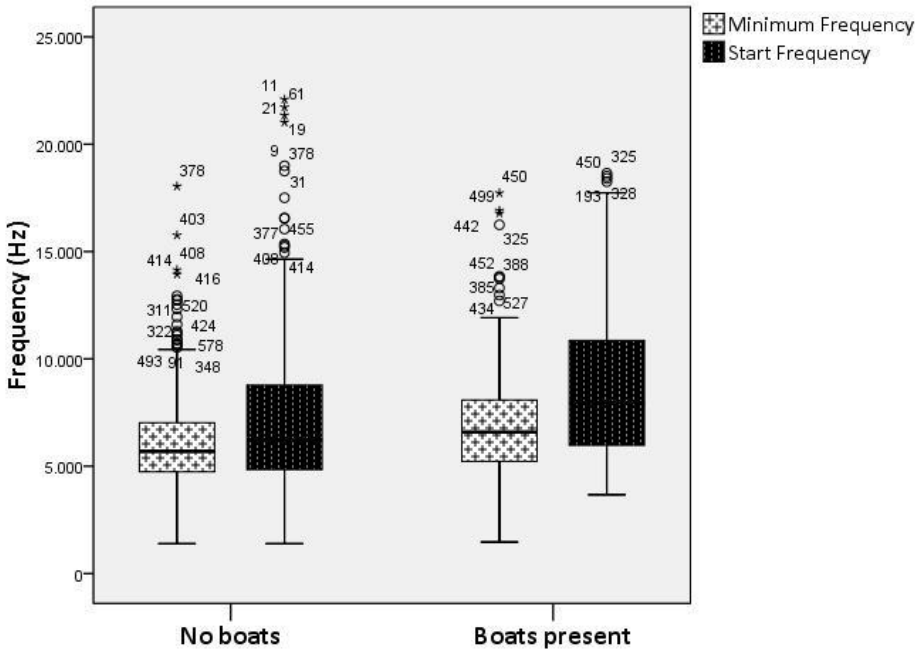


Figure 9- Mean and SE of whistles start and minimum frequency in the presence and absence of boats (The points represent outliers and asterisks represent extreme outliers).

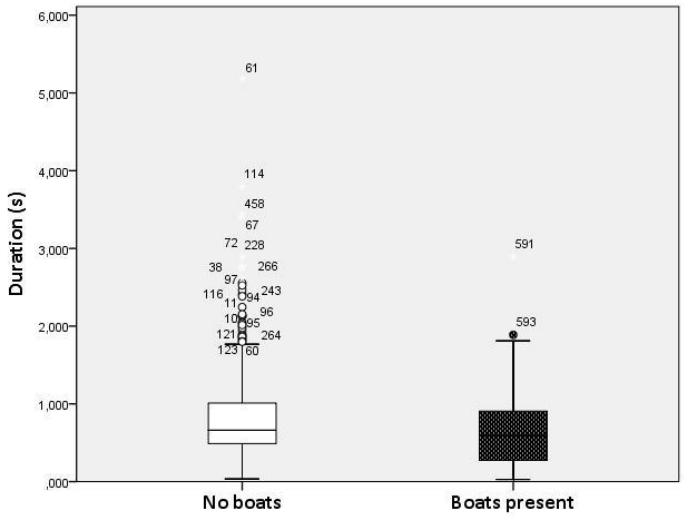


Figure 10- Mean and SE of whistles duration in the presence and absence of vessels (The points represent outliers and asterisks represent extreme outliers).

Creaks

A total of 94 creaks (no boats = 47; boats present = 47) were selected for acoustic parameter analysis. Nonparametric multivariate analyses of variance revealed statistically significant differences in the acoustic parameters rates between presence and absence of vessels (X^2 (8) = 19.53; N = 94; p = 0.012). Significant differences were found for minimum frequency (U = 1550; W = 2678; p = 0.001) and delta frequency (U = 723; W = 1851; p = 0.004). The minimum frequency was higher in the presence of vessels than in their absence (Figure). The opposite was found for delta frequencies: higher in the absence of boats than in their presence (Figure). Differences in peak frequency, maximum frequency, duration, number of pulses, ICI and repetition rate were not significant (see Table 4, in Appendix II, for more information).

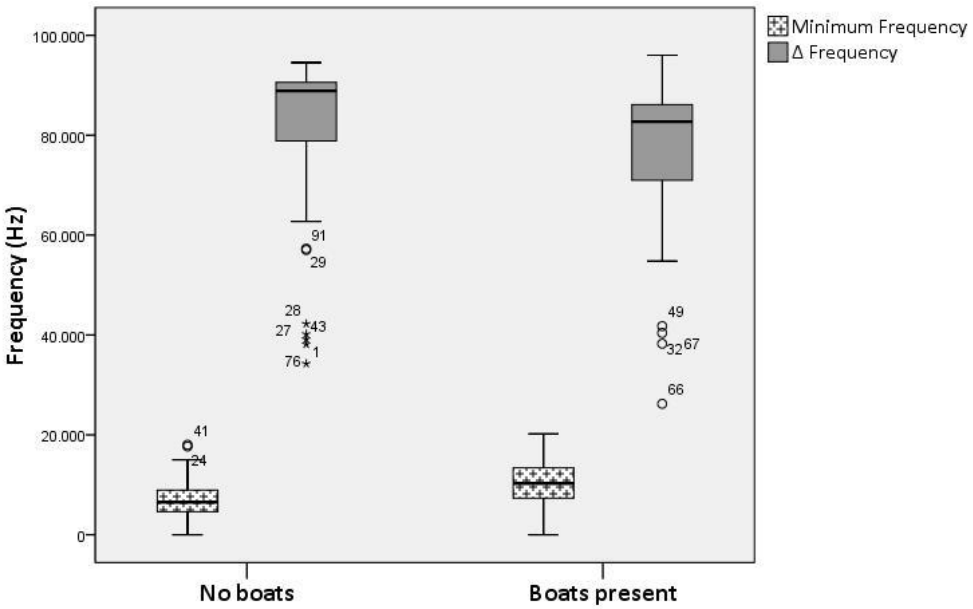


Figure 11- Mean and SE of creaks minimum and delta frequency in the presence and absence of vessels (The points represent outliers and asterisks represent extreme outliers).

Grunts

A total of 340 grunts (no boats = 208; boats present = 132) were selected for acoustic parameter analysis. Nonparametric multivariate analyses of variance showed that differences in the acoustic parameters between presence and absence of vessels were statistically significant (X^2 (8) = 80.968; N= 339; $p < 0.001$). Significant differences were found for minimum frequency (U = 9548; W = 18326; $p < 0.001$), maximum frequency (U = 8899; W = 17677; $p < 0.001$) and duration of grunts (U = 11958; W = 20736; p = 0.045). The minimum frequency was significantly lower in the absence of boats than in their presence (Figure). As for the maximum frequency, the contrary was observed: frequencies were higher in the absence of ships and lower in their presence (Figure). Grunts were longer in duration in the absence of vessels and shorter in their presence (Figure). Significant differences for the delta frequency, peak frequency, number of pulses, ICI and repetition rate were not found (more details in Table 5, in Appendix II).

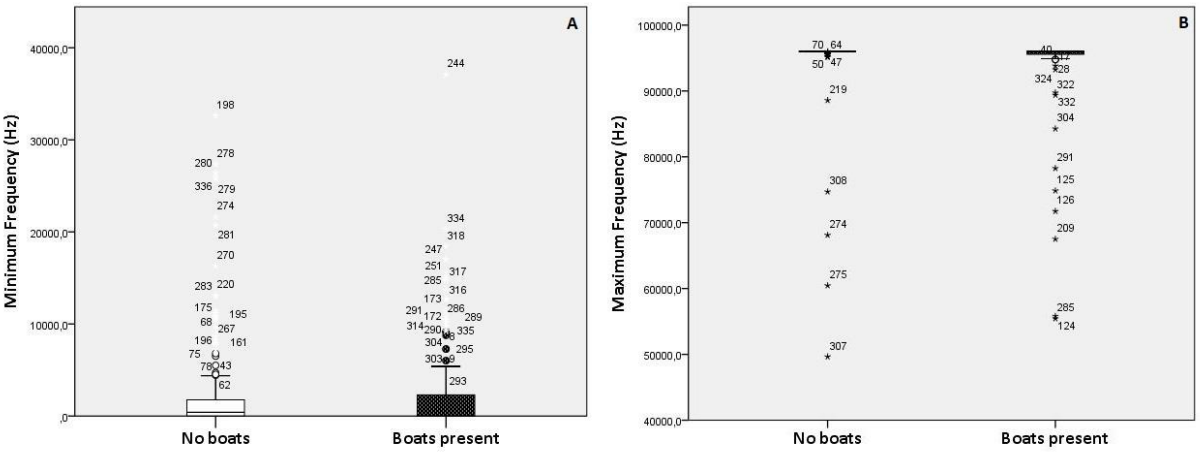


Figure 12- Mean and SE of grunts minimum (A) and maximum (B) frequencies in the presence and absence of vessels (The points represent outliers and asterisks represent extreme outliers).

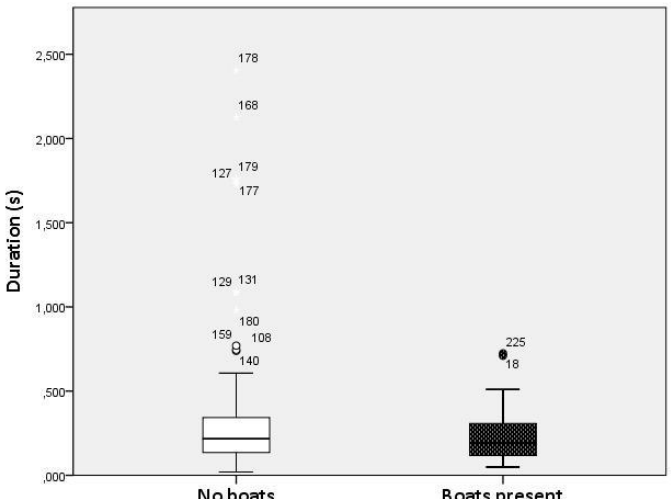


Figure 13- Mean and SE of grunts duration in the presence and absence of vessels (The points represent outliers and asterisks represent extreme outliers).

Gulps

A total of 261 gulps (no boats = 184; boats present = 77) were selected for acoustic parameter analysis. Nonparametric multivariate analyses of variance showed that differences in the acoustic parameters between presence and absence of vessels were statistically significant (X^2 (7) = 58.76; N = 260; p < 0.001). Significant differences were found for minimum frequency (U = 10401; W = 13404; p < 0.001), delta frequency (U = 3994; W = 6997; p < 0.001), peak frequency (U = 9461; W = 12464; p < 0.001), duration (U = 3715; W = 6718; p < 0.001) and end frequency of gulps (U = 9657; W = 12660; p < 0.001). In the presence of vessels, minimum frequency, peak frequency and final frequency were higher, while delta frequency and duration were shorter (Figure , Figure). Differences for maximum frequency and start frequency showed no statistical significance (see Table 6, in Appendix II, for more details).

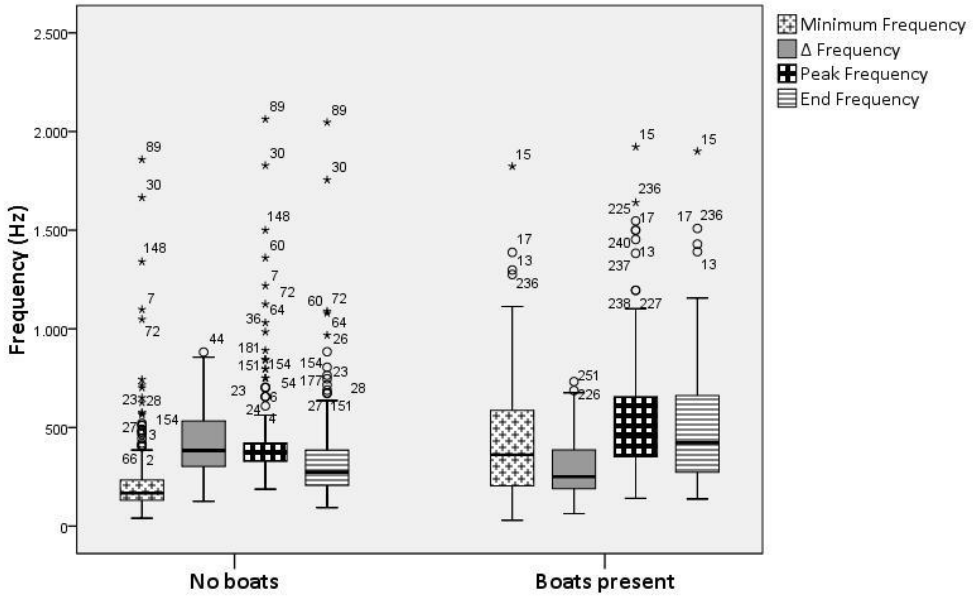


Figure 14- Mean and SE of gulps minimum, delta, peak and end frequencies in the presence and absence of vessels (The points represent outliers and asterisks represent extreme outliers).

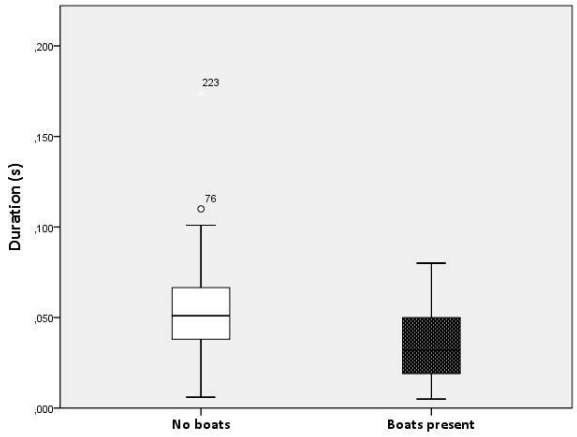


Figure 15- Mean and SE of gulps duration in the presence and absence of vessels (The points represent outliers and asterisks represent extreme outliers).

Squeaks

A total of 122 squeaks (no boats = 100; boats present = 22) were selected for acoustic parameter analysis. Nonparametric multivariate analyses of variance revealed significant differences in the acoustic parameters between presence and absence of vessels (X^2 (10) = 25.894; N = 121; p = 0.004). Significant differences were found for maximum frequency (U = 1548; W = 1801; p = 0.003), delta frequency (U = 1559; W = 1812; p = 0.002), peak frequency (U = 1498; W = 1751; p = 0.008), number of pulses (U = 1514; W = 1767; p = 0.006), ICI (U = 796; W = 1049; p = 0.043) and squeaks repetition rates (U = 1403; W = 1656; p = 0.044), were found. In the presence of boats, there was an increase in maximum frequency, delta frequency and peak frequency (Figure). There was also an increase in the number of pulses and repetition rate, and a decrease in ICI (Figure). Differences in minimum frequency, duration, start frequency and end frequency were not statically significant (for more information see Table 7, in Appendix II).

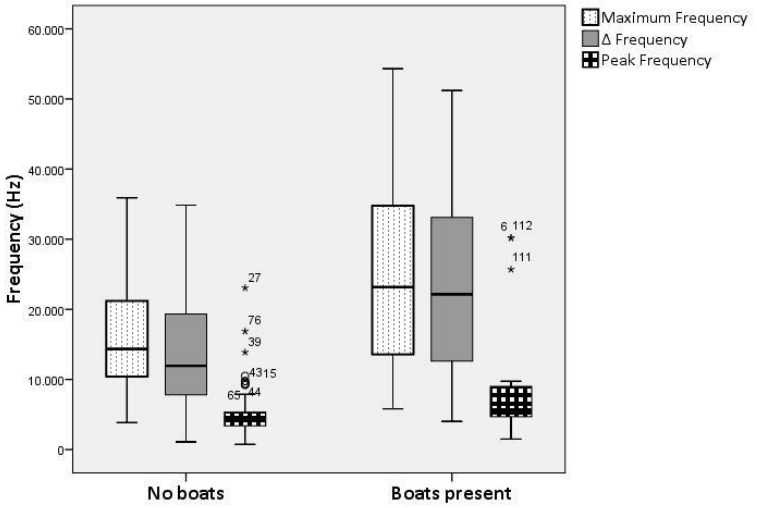


Figure 16- Mean and SE of squeaks maximum, delta and peak frequencies in the presence and absence of vessels (The points represent outliers and asterisks represent extreme outliers).

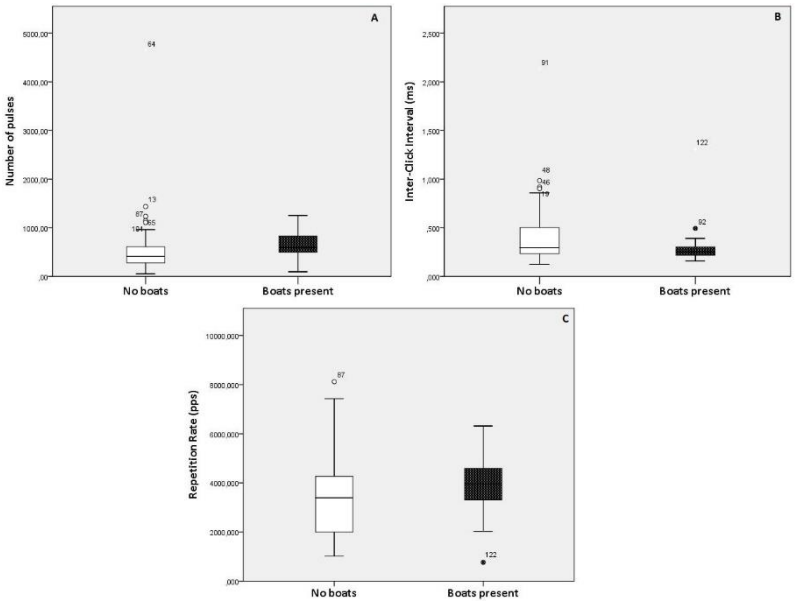


Figure 17- Mean and SE of squeaks number of pulses (A), inter-click intervals (B) and repetition rate (C), in the presence and absence of vessels (The points represent outliers and asterisks represent extreme outliers).

Squawks

A total of 152 squawks (no boats = 81; boats present = 71) were selected for acoustic parameter analysis. Nonparametric multivariate analyses of variance revealed no significant differences in the acoustic parameters between presence and absence of vessels (X^2 (8) = 10.117; N = 152; p = 0.257).

Slow-click trains

A total of 296 slow-click trains (no boats = 157; boats present = 139) were selected for acoustic parameter analysis. Nonparametric multivariate analyses of variance showed that differences in the acoustic parameters between presence and absence of vessels were not statistically significant (X^2 (5) = 10.03; N = 296; p = 0.074).

Overall, differences in acoustic parameters between vessels presence and absence were found for the following vocal elements: whistles, creaks, gulps, grunts and squeaks. However, no differences were found, for squawks and slow-click trains. The mean call rate for each vocal type also showed no significant differences.

DISCUSSION

In this study, the acoustic behavior of bottlenose dolphins' resident in the Sado estuary was recorded in the absence and presence of vessels operating within a 1000-m radius.

The noise created by vessels below 100 Hz was not analyzed in this work, due to the 100 Hz high-pass filter used in recordings. This high-pass filter helps minimize the noise generated by the movement of water masses and self-noise generated by the research platform and other low-frequency vibrations such as wind and surface agitation. However, the low frequency noise has probably little effect on bottlenose dolphins, as they have a reduced hearing sensitivity for frequencies below 100 Hz (Richardson *et al*., 1995). Furthermore, in the Sado estuary, the majority of ships, from medium to large sized, create most noise around 400 Hz (Cruz, 2012; Luís *et al*., 2012). Other vessels dominate medium frequencies bands, between 2.5 kHz and 10 kHz (Cruz, 2012). A typical cargo ship entering the Sado estuary generates a pressure change (the measurable noise) of approximately 180 dB re 1 µPa @ 1m (dos Santos, 1998; Richardson *et al*., 1995) using a standard cylindrical propagation model, at a distance of 1000 m, the noise level will still be in the range of 140 dB re 1µPa, therefore with potential for masking dolphins vocalizations of similar frequencies (Luís *et al*., 2012).

Emission rates

If each individual maintained its constant emission rate, variations in call rate would be proportional to group size. However, the emission rates did not vary with group size, as expected, considering previous studies (dos Santos *et al*., 2005; Luís *et al*., 2012). This could be explained by the fact that dolphins in large groups tend to reduce emissions per individual, to avoid mutual masking of signals and to enssure transmission of information (Quick & Janik, 2008).

Although differences in mean call rate have been found in the past within this population (Luís *et al*., 2012, 2014) this study did not detect significant differences in mean call rate for any of the vocal elements. Nonetheless, variations in call rate (increase or decrease) in the presence of noise have been reported in other studies (Buckstaff, 2004; Lesage, 1999; Luís *et al*., 2014; Weilgart 2007). However, this is not the first time that it is documented that cetaceans do not change their phonation rates as a result of vessel noise (Lemon *et al*., 2006; Foote *et al*., 2004).

An explanation for this fact may be that dolphins in Sado estuary are more tolerant or habituated to vessel noise. Other cetaceans have shown apparent tolerance to anthropogenic noise, by not altering their emission rates and by their continued presence in some major shipping routes and fishing grounds (Weilgart, 2007). This population has been studied in the area since the 1980s and it is known that the estuary is an important feeding ground for these animals (dos Santos *et al*., 2007; dos Santos & Lacerda, 1987).

Many odontocetes often tolerate repeated exposure to vessel traffic noise (tolerance refers to the occurrence of animals in areas where they are exposed to anthropogenic noise) (Richardson *et al*., 1995). Marine mammals may show short-term behavioral reactions and localized or temporary displacement when they are exposed to particularly strong disturbance and still be tolerant to the noise source (Richardson *et al*., 1995). In cetaceans, habituation certainly was involved in developing tolerance to some types of noise and disturbance, to which animals are repeatedly exposed (Richardson & Würsig, 1997).

The fact that there are no differences in mean call rate suggests that the population is habituated to vessel noise. In past studies, the population showed several behavioral responses to ship noise, including differences in mean call rate of creaks, whistles and squawks (Cascão,

2001; Luís, 2007; Luís *et al*., 2012, 2014). In this study, data from 2014 to 2017 was used and variations in emission rates were not found which could mean that dolphins are less responsive to overall ship noise.

Whistles

Significant changes were observed in whistle characteristics: in the presence of boats, dolphins emitted shorter whistles with higher minimum and start frequency. These results are in agreement with previous studies conducted in this population (Luís *et al*., 2014; Rocha, 2012).

Due to spectral overlap, boat noise is likely to reduce range at which dolphin whistles can be heard by conspecifics, and has the ability to mask signals (Buckstaff, 2004; Jensen *et al*., 2009; Richardson, 2012). As such, shifts in frequency of whistles are a common short-term response of marine mammals to noise, in order to increase signal detectability or compensate masking effects (Cruz, 2012; Foote *et al*., 2004; Guerra *et al*., 2014; Jensen, 2009; Jensen *et al*., 2012; Lesage *et al*., 1999; Luís *et al*., 2014; Papale *et al*., 2015; Rocha, 2012).

Vessel noise therefore can affect whistle characteristics, and the resident dolphins seem to need to shift their frequencies upwards to be able to communicate in noisier circumstances. The behavioral and social consequences of these specific changes are presently difficult to evaluate.

Variations in call duration have also been observed in many studies as a short-term response to noise (Bittencourt *et al*., 2016; Guerra *et al*., 2014; La Manna *et al*., 2013). This population produced shorter signals in the presence of boats, contrary to what most studies have observed, though this was not the first case this response was observed (Foote *et al*., 2004; Lesage, 1999). According to Bittencourt *et al*. (2016), a possible explanation for shorter signals is the energy cost associated with increasing frequency and duration at the same time. Furthermore, shorter signals increase the reception probability in the presence of intermittent noise sources, such as boat traffic, and could be an important mechanism to avoid masking.

Noise-induced vocal responses have biological costs that include increased detection by predators or competitors, degraded signal efficacy or function in social contexts, as well as, energetic costs related to changes in metabolic demands or activity budgets (Holt *et al*., 2015). Increases in the amplitude, duration and/or repetition rate of acoustic signals have metabolic consequences (Holt *et al*., 2015). Producing shorter signals with higher frequencies may be the less energy demanding strategy for these dolphins in response to vessel noise.

Creaks

Significant changes were observed in creaks characteristics: in the presence of boats, dolphin's minimum frequency was higher and bandwidth was shorter which is expected when dolphins shift their minimum frequency to higher values.

Creaks are burst-pulses often emitted during echolocation/targeting in foraging and feeding events and could represent a cue for feeding to conspecifics in the area (Madsen *et al*., 2007; Miller *et al*., 2004). In previous studies, emission of creaks was lower in the presence of vessels, which suggests impacts in foraging activities (Aguilar Soto *et al*., 2006; Luís *et al*., 2014).

As mentioned above, shifting to more elevated frequencies in the presence of vessels seems to be a mechanism to avoid masking, resulting in more efficient foraging and feeding activities, in a noisy background.

Although burst pulses are formed by broadband pulses often similar to echolocation signals, there seems to be a distinction between the signal types associated with bio-sonar tasks (like creaks and slow-click trains) and other pulsed signals that probably have a short-distance

communication function (Luís *et al*., 2016; Lammers *et al*. 2003). However, information regarding properties and function of burst pulses is scarce.

Grunts

Differences in acoustic parameters of grunts were also significant: in the presence of boats, dolphins emitted shorter grunts with higher minimum and maximum frequencies.

Grunts are burst pulses, with strong emphasis in the lower frequencies. Since the main energy of the grunts occurs within the boat noise spectrum it is expected that vessel noise masks these signals (Simard *et al*., 2011). Grunts have been described as aggressive calls in agonistic encounters, and may be linked to intraspecific competition for prey (Bass & Clark, 2003; Blomqvist & Amundin, 2004; Luís & dos Santos, 2012).

Variations in grunt acoustic frequency seems to be linked to counterbalance masking and may have an important role in competition for prey in feeding and foraging contexts. Since finding and capturing prey is likely hampered by vessel noise, competition in the presence of boats is likely to increase. Therefore, dolphins probably shift their frequencies as to make themselves heard by conspecifics in competition for prey. Again, producing shorter signals, might be more efficient, a speculation that follows from the present results.

Gulps

In the presence of vessels, gulps had significant acoustic differences: dolphins produced shorter gulps with higher frequencies.

Low frequency narrow-band sounds, like gulps, may function predominantly as a short-range call (Simard *et al*., 2011). It is likely that vessel noise has a large impact on gulps detectability, and, as such, shifting to higher frequencies is a mechanism to overcome masking. Furthermore, gulps are often found in bray-series and have been often reported during sexual, social or agonistic situations in dolphins (dos Santos *et al*., 1995; Simard *et al*., 2011). So, changes in frequencies may have consequences in the social context.

The mechanism of production and energetic cost of these signals are unknown (dos Santos *et al.*, 1995). Nonetheless, knowing that shifts to a higher frequency have metabolic costs and producing shorter signals seems to be what is more efficient for this population, as explained earlier.

Squeaks

Squeaks showed significant differences in the presence of vessels, such as, higher peak and maximum frequency, and consequently, an increase in bandwidth. Additionally, in the presence of vessels, there was an increase in the number of pulses and repetition rate, and a decrease in inter-click interval.

Very high pulse repetition rate bursts and short inter-click intervals (< 10ms), like those observed in squeaks, may have a communicative function and not serve the purposes of echolocation, considering that dolphins may not process the returning echoes from click trains with very short intervals (Blomqvist & Amundin, 2004; Cranford, 2000; Lammers, 2003). Besides, differences in inter-click intervals may be linked to behavioral state (Lammers *et al*., 2003).

It would seem that increases in frequencies in this vocal element have the function of maintaining communication against induced masking by vessel noise. As far as differences in inter-click intervals, variations may be a result of behavioral state of dolphins in the presence of boats. Differences in number of pulses could be a direct result of the decrease in ICI, since these concepts are highly linked. Although it is not possible to determine the biological meaning of these observed changes, they show that vessel noise has an effect on this type of sound.

Bray-series, as mentioned before, are rhythmic, sequences containing gulps alternated with squeaks and/or grunts (dos Santos *et al*., 1995). Brays are often recorded in highly social contexts such as agonistic encounters and feeding contexts, and only in some populations so their primary function is likely communicative rather than echolocation (dos Santos *et al*., 1995; Janik, 2000; King & Janik, 2015; Lammers, 2003; Luís & dos Santos, 2012). Bray-series emitted during feeding activity might serve as conspecifics calls but could also be used to modify prey's behavior by stunning (dos Santos *et al*., 1995; Janik, 2000; King & Janik, 2015; Luís & dos Santos, 2012;). Besides, variability in brays sequences might be related with the motivational state of the emitter, being an indicator of arousal or excitement (dos Santos *et al*., 1995).

All things considered, variations in acoustic parameters in the presence of vessels, such as grunts, gulps and squeaks, might serve the purpose of maintaining communication between the group during foraging and feeding events. They may also indicate competition for prey, which could explain the agonistic behavior and presence of these signals during feeding activities.

Squawks

Squawks have been documented combined with several variants in different contexts, such as affiliative, agonistic, aggressive, sexual, feeding and foraging contexts (Herzing, 1968 in Au & Hastings, 2008; Lilly & Miller, 1961; Luís et al., 2012, 2016; Overstrom, 1983) and, more recently in food reward context (Ridgway et al., 2014).

In squawks, no significant differences were found regarding presence of vessels. This could be due the high frequencies of which squawks are emitted, peak frequency above 20 kHz (more details in Table 8, in Appendix II). Squawk frequencies seem to be high enough to not be disturbed by boat noise, since changes in acoustic parameters are a response to masking, and in this case, this was not observed.

Most shipping produces noise in the low frequency range (less than 1 kHz) but some small leisure craft noise sound from 1 kHz up to 50 kHz (Parsons, Swift & Dolman, 2003). Since peak frequency of squawks is above 20 kHz, it appears that this type of signal is not masked by boat noise, and dolphins do not have to change their vocalization.

Slow-click trains

In this study, there was no significant change in acoustic parameters of slow click trains according to ship noise.

Echolocation clicks are used by dolphins to perceive their environment and to detect prey, predators and obstacles (Au, 1993). Travelling groups of resident dolphins are likely to be familiar with their environment and conspecifics (Lemon, 2006). As such, they may be able to navigate more efficiently, while producing fewer clicks (Lemon, 2006). In the Sado estuary, the bottlenose dolphin resident population spends a great deal of time in limited area, and has for a long time. Therefore, bottlenose dolphins might be able to navigate in the estuary without having to emit echolocation clicks, since they are already familiarized with the environment. This could also explain why in previous studies no alterations were recorded in emission rates of

slow-click trains in the presence or absence of boats, and why global emissions of this element are lower than expected (dos Santos & Almada, 2004; Luís *et al*., 2014;).

These results indicate modifications in acoustic behavior in the presence of vessels, suggesting that this bottlenose dolphin population, while showing some tolerance, may be disturbed by noise generated from boats in their habitat. Furthermore, this study shows that dolphins in the Sado estuary adapt their vocal frequencies and produce shorter signals probably as a compensatory strategy to maintain levels favorable to communication. Such vocal compensation might be significant, as increasing vocal output to compensate for noise has energetic costs, and other behavioral or social effects are unknown.

It is important to notice that there is an inconsistence use of acoustic terminology regarding odontocetes vocalizations (Au, 2000). Since a standard nomenclature does not exist, similar sounds are often given different names (e.g. squawks (Herzing, 1996) are termed creaks in this population; victory squeals (Ridgway *et al*., 2014) are squawks and feeding buzzes (Ridgway *et al.*, 2014) are creaks). This makes comparisons between sounds difficult and confuses homologies. The pulsed signals category still remains the least understood, in structure, function and significance, which urges the need to improve accuracy of categorizations and a standard nomenclature.

CONCLUSION

1. General conclusion

There are many cases of cetaceans remaining in high-noise environments for prolonged periods, probably motivated by food availability and, which seems to be the case of the resident bottlenose population in Sado estuary. This study shows evidence that dolphins resident in Sado estuary are tolerant to ship noise, possibly an effect of habituation, since it appears to be a change in response to noise.

Marine mammals are known to adjust their acoustic behavior to avoid masking, by changing call emission rates, duration and/or by shifting the acoustic frequency of vocal elements. Evidence of this mechanism was observed in this study, for whistle, creaks, grunts, gulps and squeaks. Considering that the low frequency acoustic signals and vessel noise overlap, bray series, gulps, grunts and squeaks, are potentially at risk of significant masking by anthropogenic noise. This should have serious impacts in foraging and feeding events, since these calls seem to be linked to these functions. This suggests that vessel noise has an impact on these animals and could have metabolic consequences, even though they seem to tolerate it, responding with adaptive adjustments.

The biological consequences of acoustic responses to noise in cetaceans are difficult to ascertain. Long term consequences cannot be estimated without knowing how vocal response affects energy budgets, the importance of production of pulsed signals and other possible social interaction consequences.

The bottlenose dolphins in the Sado estuary form one of the few resident populations in Europe, and the only one in Portugal. This small and aged population suffers impacts of noise produced by ships, and efforts to preserve these animals should be made to assure that dolphins continue to live in this habitat. The species itself is protected, and it uses two protected areas - this should be taken into consideration in the special conservation plans, current and future.

2. Future studies

This seems to be the first study to evaluate variations in acoustic parameters of different vocal elements, including burst pulses, such as creaks, grunts, gulps and squeaks.

The understanding of functional contexts and production of burst pulse signals is needed, in order to estimate the real impacts of vessel noise in these animals. It seems that these signals are of importance to these species, making them shift to higher frequencies to convey information or maintain contact. Future research regarding impacts of anthropogenic noise should include these vocal elements, and bray-series. It would be interesting to evaluate modifications and compare results between populations.

Long-term studies are of major importance, since such impacts would probably only be visible after years of extensive population monitoring. Therefore, this population must be monitored in close range, and future studies should continue to evaluate the effects of vessel noise on their behavior.

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APPENDIX

Appendix 1. - Sado estuary Bottlenose Dolphins Vocal Repertoire *(Tursiops truncatus)*

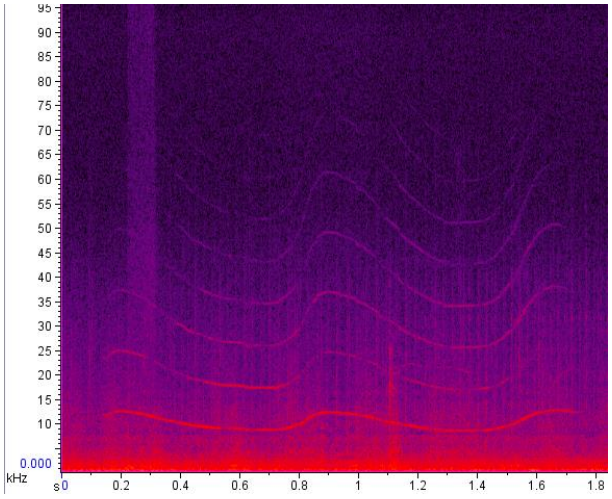

Figure 18- Example of sonogram of whistle

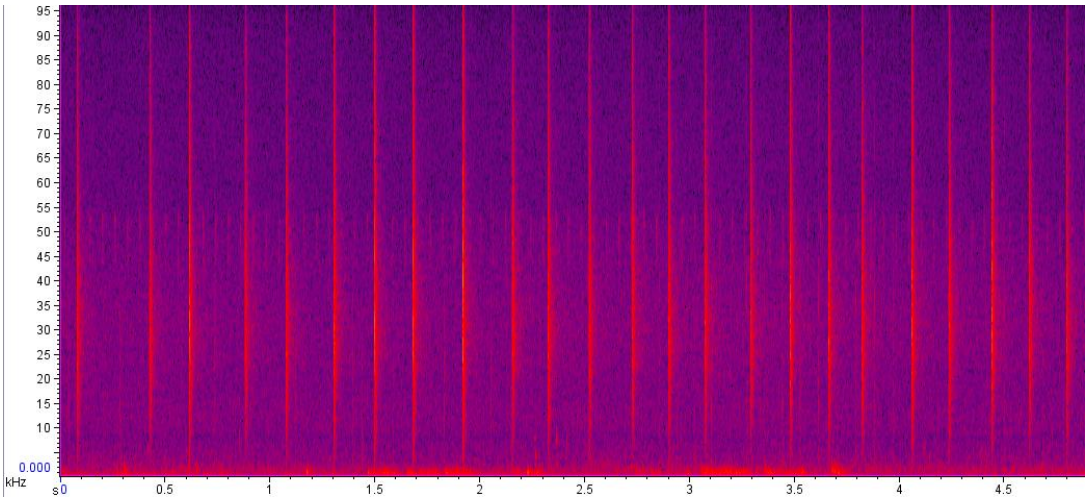

Figure 19- Example of sonogram of slow-click train

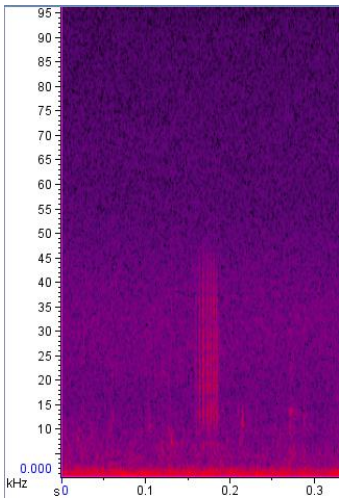

Figure 20- Example of sonogram of short-burst pulse

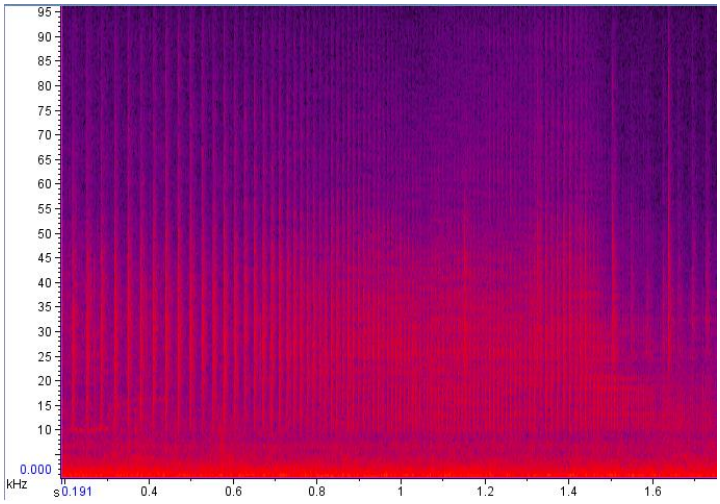

Figure 21- Example of sonogram of creak

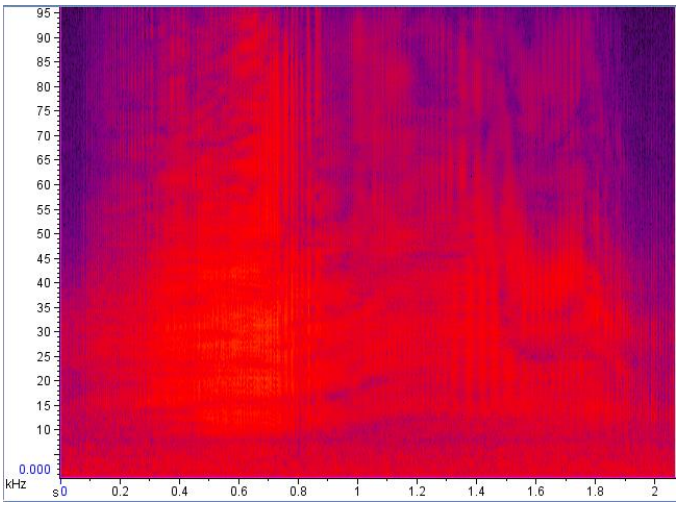

Figure 22- Example of sonogram of squawk

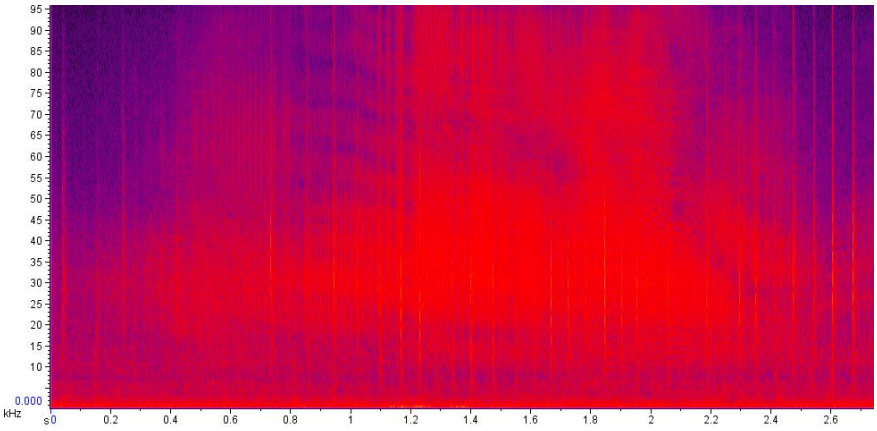

Figure 23- Example of sonogram of TTV

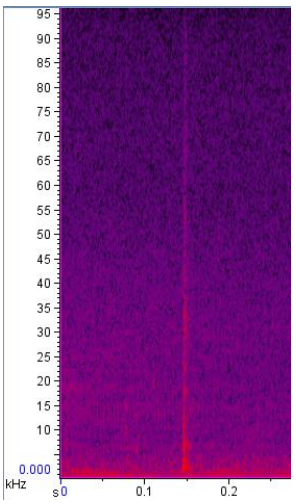

Figure 24- Example of sonogram of bang

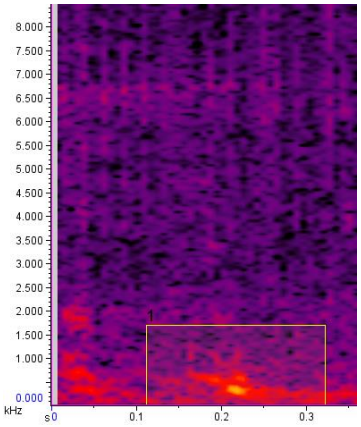

Figure 25- Example of sonogram of gulp

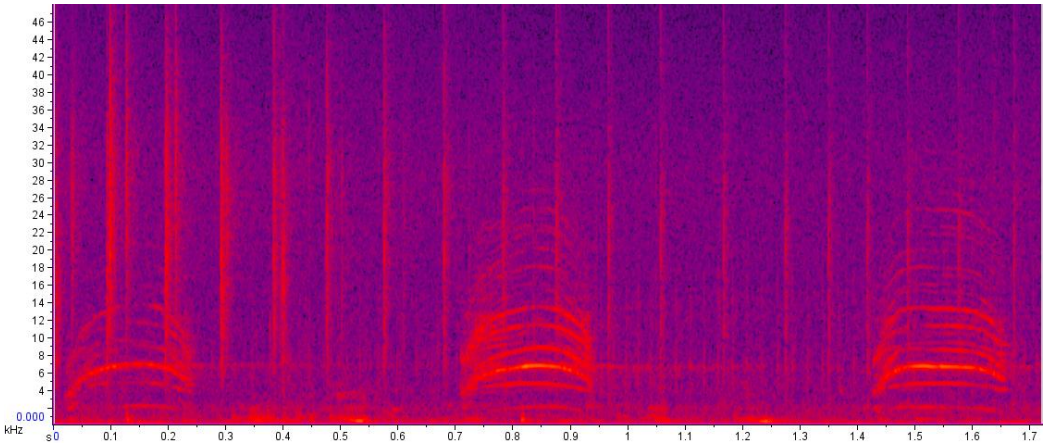

Figure 26- Example of sonogram of squeak

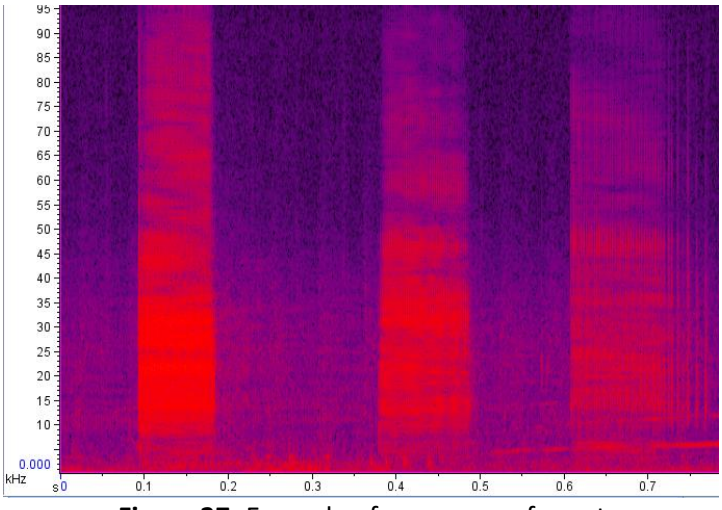

Figure 27- Example of sonogram of grunt

Appendix 2. -Mean values and SE

Table 2- Mean values and SE of emission rates in the presence and absence of boats

		bang	short-burst pulses	slow-click trains	creak	squawk	TTV	whistle	grunt	gulp	squeak
Absence of vessels	Mean	0.199	0.035	5.611	0.449	0.620	0.570	2.759	1.487	1.103	0.888
	SE	0.073	0.016	0.428	0.132	0.118	0.146	0.508	0.375	0.383	0.240
Presence of vessels	Mean	0.042	0.035	5.690	0.613	0.642	0.480	2.008	1.093	0.620	0.366
	SE	0.028	0.025	0.655	0.173	0.160	0.147	0.528	0.372	0.272	0.180

Table 3- Mean values and SE of whistles parameters in the presence and absence of boats

		Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Start Frequency (Hz)	End Frequency (Hz)	Duration (s)	Inflexion Points
Absence of vessels	Mean	6099.53***	15457.26	10279.63	7233.10***	11898.92	4.627*	1.13
	SE	111.24	176.35	164.72	176.87	229.11	3.808	0.08
Presence of vessels	Mean	6975.38***	15643.15	10673.61	8778.24***	12631.09	0.650*	1.28
	SE	176.99	259.57	261.90	239.69	310.63	0.030	0.13

The mean difference is significant at the *< 0.05, ** < 0.01, *** < 0.001 level.

Table 4- Mean values and SE of creaks parameters in the presence and absence of boats

		Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Δ Frequency (Hz)	Duration (s)	No. of pulses	ICI (ms)	Repetition Rate (pps)
Absence of vessels	Mean	7208.80***	87749.57	24191.49	80540.76**	0.431	54.30	0.0096	146.05
	SE	595.81	2329.29	1349.23	2498.61	0.030	3.90	0.0010	13.74
Presence of vessels	Mean	10159.38***	86818.43	26904.26	76659.06**	0.396	46.30	0.0111	131.96
	SE	664.19	2365.32	1283.04	2267.16	0.021	3.52	0.0011	13.34

The mean difference is significant at the *< 0.05, ** < 0.01, *** < 0.001 level.

Table 5- Mean values and SE of grunts parameters in the presence and absence of boats

		Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Δ Frequency (Hz)	Duration (s)	No. of pulses	ICI (ms)	Repetition Rate (pps)
Absence of vessels	Mean	2428.30***	95290.33***	11793.48	92862.06	0.295*	70.65	0.0040	300.14
	SE	344.95	328.39	932.26	487,16	0.022	3.53	0.0002	7.72
Presence of vessels	Mean	2856.14***	94254.23***	13832.39	91398.16	0.225*	64.84	0.0036	314.38
	SE	506.31	552.15	1374.05	756.40	0.012	3.15	0.0001	9.40

The mean difference is significant at the *< 0.05, **< 0.01, ***< 0.001 level.

Table 6- Mean values and SE of gulps parameters in the presence and absence of vessels

		Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Δ Frequency (Hz)	Duration (s)	Start Frequency (Hz)	End Frequency (Hz)
Absence of vessels	Mean	245.77***	667.47	421.70***	429.13***	0.053***	558.15	342.54***
	SE	17.78	21.48	12.10	18.74	0.002	18.58	17.91
Presence of vessels	Mean	455.15***	754.31	299.16***	601.16***	0.034***	675.81	531.82***
	SE	40.53	45.21	17.45	43.69	0.002	45.76	41.31

The mean difference is significant at the *< 0.05, ** < 0.01, *** < 0.001 level.

Table 7- Mean values and SE of squeaks parameters in the presence and absence of vessels

		Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Δ Frequency (Hz)	Duration (s)	No. of pulses	ICI (ms)	Repetition Rate (pps)	Start Frequency (Hz)	End Frequency (Hz)
Absence of vessels	Mean	2450.14	16292.92**	4925.63**	13842.77**	0.155	511.09**	0.0004*	3288.95*	4139.58	3892.90
	SE	114.94	793.24	313.59	820.27	0.007	48.52	0.00003	146.27	142.19	123.06
Presence of vessels	Mean	2018.49	25055.70**	8667.61**	23037.21**	0.159	634.05**	0.0003*	3898.08*	4076.90	3848.35
	SE	261.85	2863.81	1808.73	2824.03	0.010	61.11	0.00005	265.29	237.89	206.37

The mean difference is significant at the *< 0.05, ** < 0.01, *** < 0.001 level.

Table 8- Mean values and SE of squawks frequencies in the presence and absence of vessels

		Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)
Absence of vessels	Mean	7203,20	20574,07	89377,50
	SE	615,97	1350,84	1619,53
Presence of vessels	Mean	8067,16	24607,40	91934,10
	SE	620,15	1307,06	1156,82

Appendix 3. - Example of a sampling form

SAÍDA Nº _____ H INÍCIO _____ BM _____ METEO _____

DATA _____ H FIM _____ PM _____

ESFORÇO _____

	HORA	LATITUDE (N)		LONGITUDE (W)		PROF. (m)	GOLFINHOS			ACTIVIDADE DOMINANTE	Registar nº de embarcações e tipologia			OBSERVAÇÕES
							Dist (m)	Azimute (°)	nº individuos		20m	100m	200m	
1										A BP D S R				
2										A BP D S R				
3										A BP D S R				
4										A BP D S R				
5										A BP D S R				
6										A BP D S R				
7										A BP D S R				
8										A BP D S R				